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FIG. 1A**2H7scFv-Ig cDNA and predicted amino acid sequence:**

HindIII NcoI 2H7 V_L Leader Peptide→

1 AAGCTTGCCG CC M D F Q V Q I F S F L L I S A S
ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCCTCA

2H7 V_L→
V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT
P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI

181 Y Q Q K P G S S P K P W I Y A P S N L A
TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT
S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC
S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly₄Ser)₃ Linker
P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTCTG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H→
G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG
R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC
N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT
P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA
D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGGC
V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTCTT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

FIG.1B

BclI
-----human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H
GGCACAGGGA CCACGGTCAC CGTCTCT**GAT** CAGGAGCCCA AATCTTGTGA CAAA**ACT**CAC

841 T C P P C P A P E L L G G P S V F L F P
ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCA**CAT**G CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S
CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S
GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R
AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S
GAACCACAGG TGTACACCCT GCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F
GGGCAGCCGG AGAACA**ACTA** CAAGACCAGC CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

1381 F L Y S K L T V D K S R W Q Q G N V F S
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441 C S V M H E A L H N H Y T Q K S L S L S
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

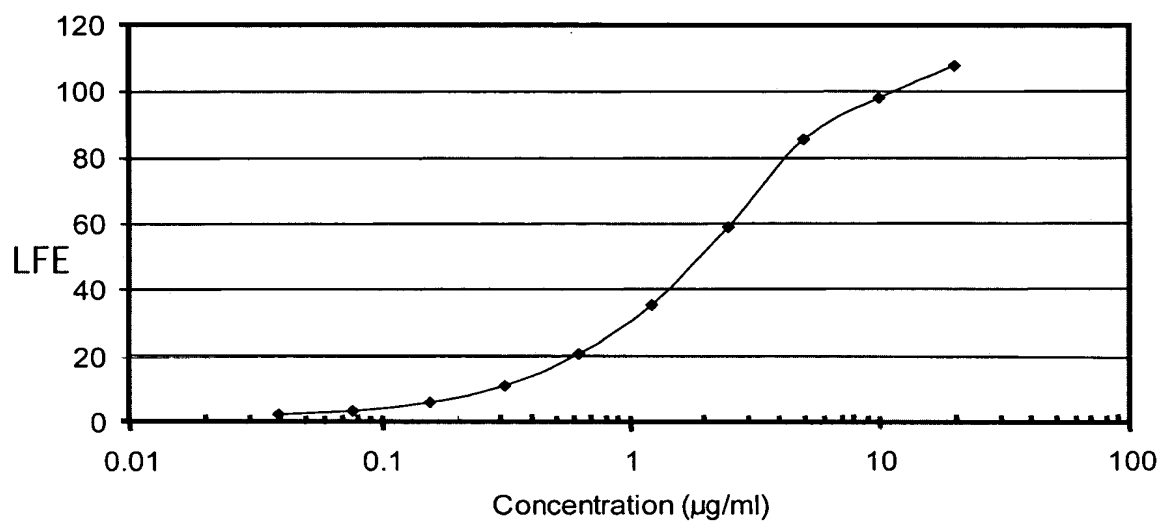
XbaI

1501 P G K * S R
CCGGGTAAAT GATCT**AGA**

FIG.2

Production Levels of 2H7 scFv (SSS-S)H WCH2 WCH3
by Stable CHO Lines

2H7 scFv (SSS-S)H WCH2 WCH3 STANDARD CURVE



Clone	LFE @ 1:50 Estimated Concentration (mg/ml)
D2	26.156
IIIC6	25.755
IVA3	28.661
Spent bulk	29.664

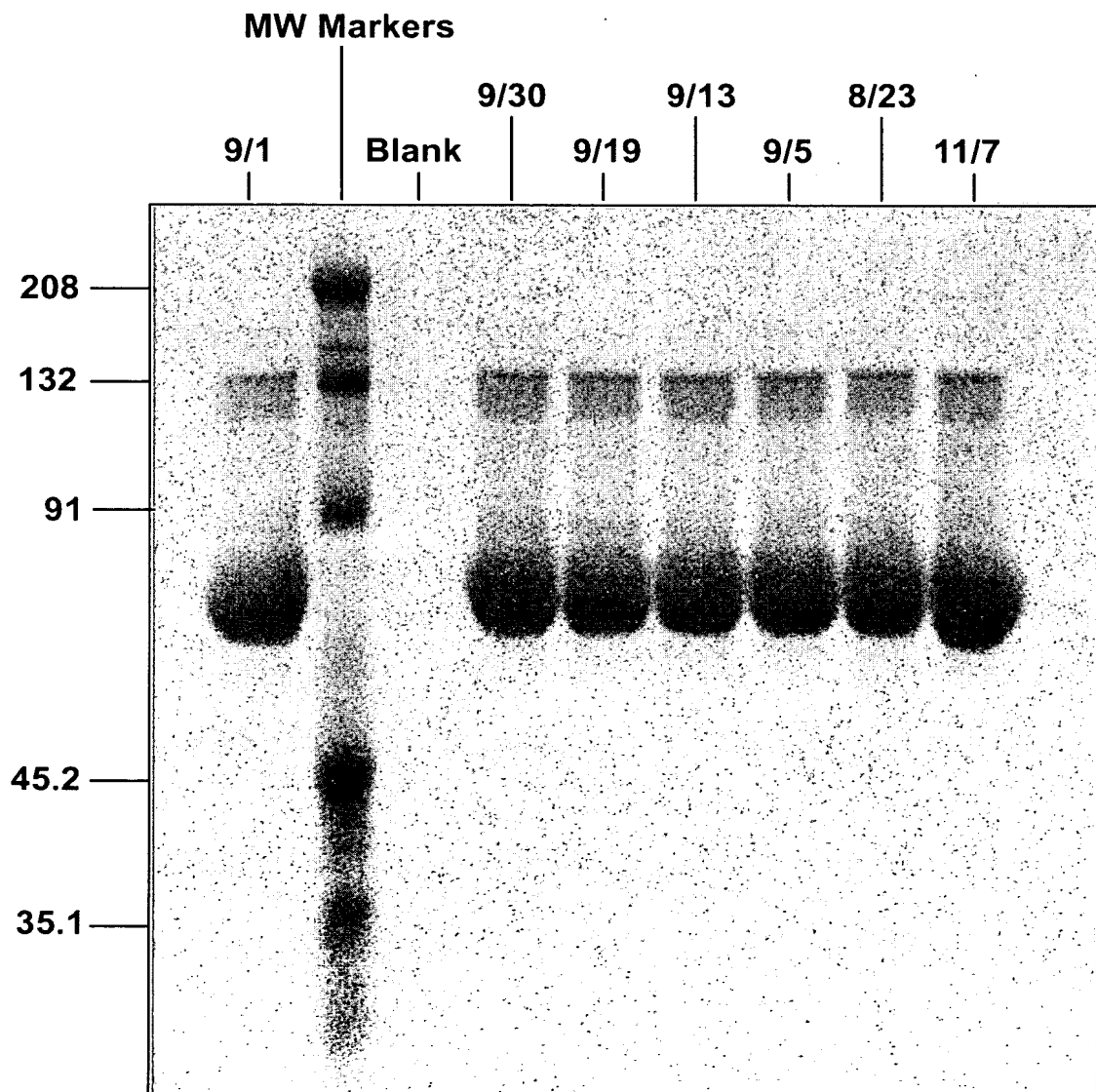
FIG.3**SDS-Page Analysis of
2H7 scFv (SSS-S)H WCH2 WCH3 Protein**

FIG.4A

**Complement Mediated B Cell Killing After Binding of CD20-targeted
2H7 scFv (SSS-S)H WCH2 WCH3**

2H7scFv-Ig Concentration		RAMOS # LIVE CELLS / TOTAL CELLS		BJAB # LIVE CELLS / TOTAL CELLS
20 µg/ml + complement	—	0.16	—	0.07
5 µg/ml + complement	—	0.2	—	N.D.
1.25 µg/ml + complement	—	0.32	—	0.1
Complement alone	—	0.98	—	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

FIG.4B

**Antibody-dependent cellular cytotoxicity (ADCC) mediated by
2H7 scFv (SSS-S)H WCH2 WCH3**

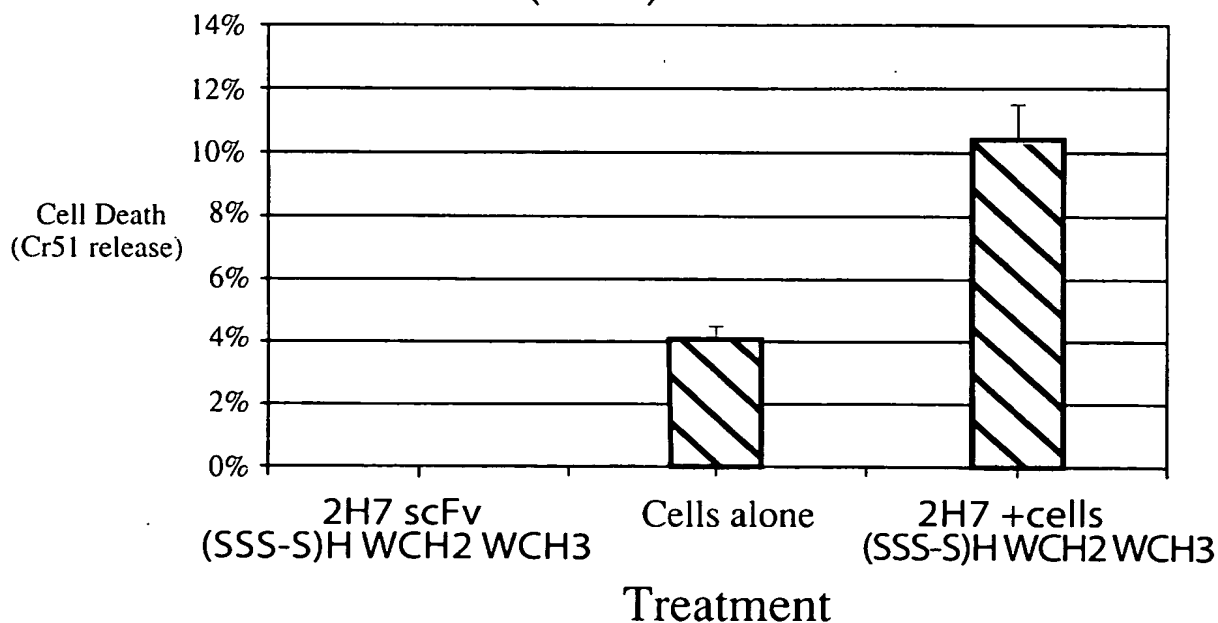
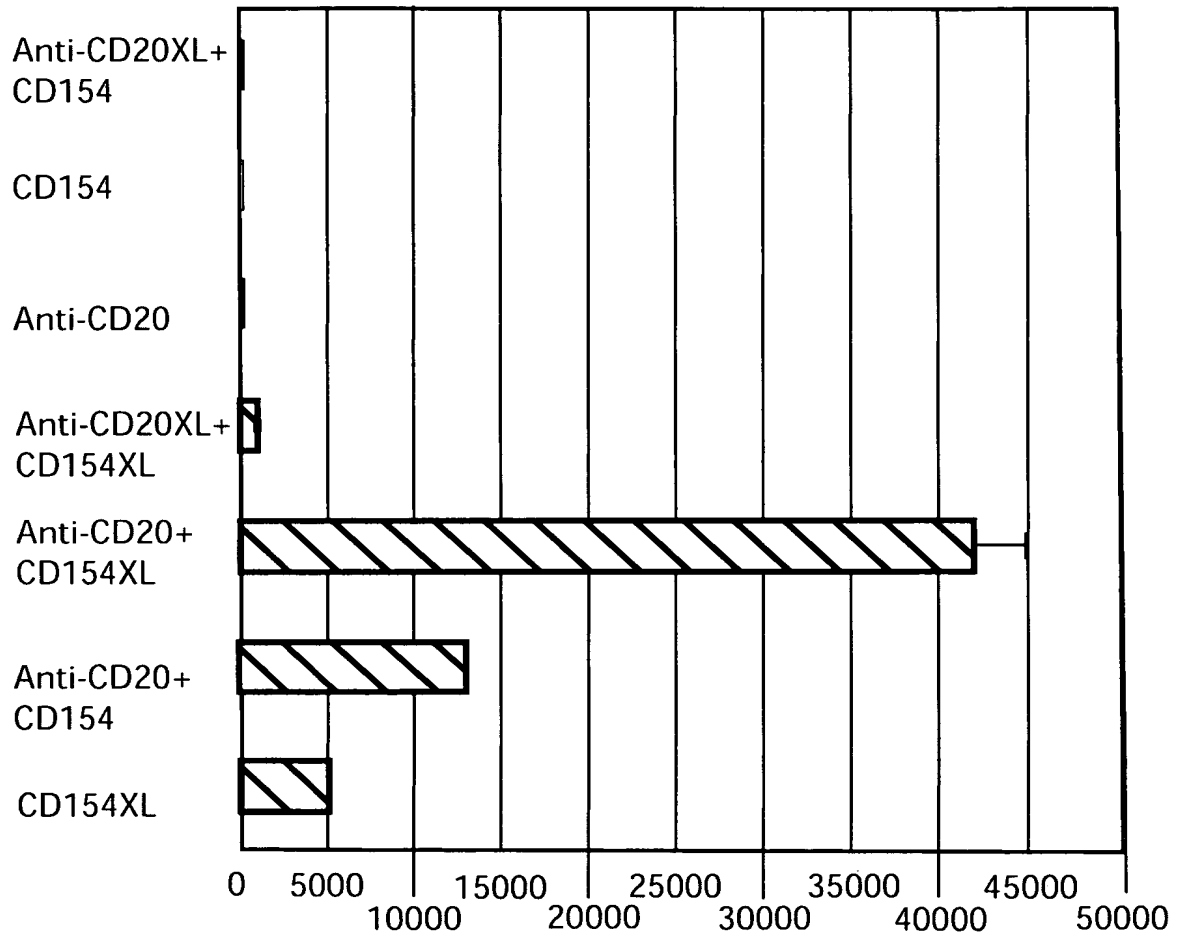


FIG. 5

Effects of Crosslinking of CD20 and CD40 Cell Surface Receptors
on B Cell Proliferation:



Counts per minute (CPM) INCORPORATED

FIG.6A

Effect of Simultaneous ligation of CD20 and CD40 on CD95 and apoptosis.

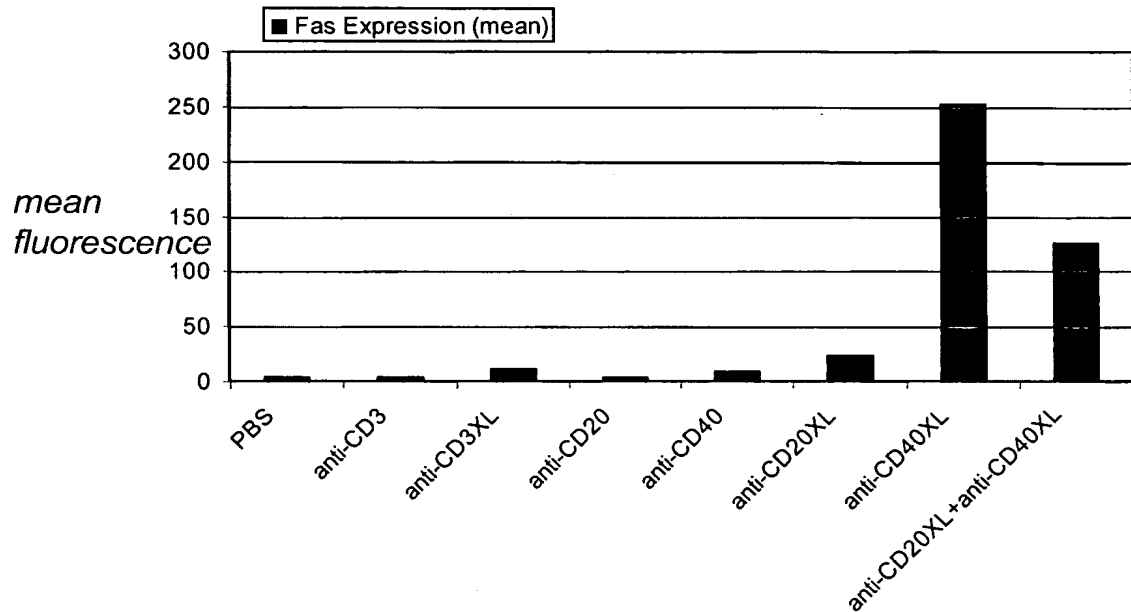
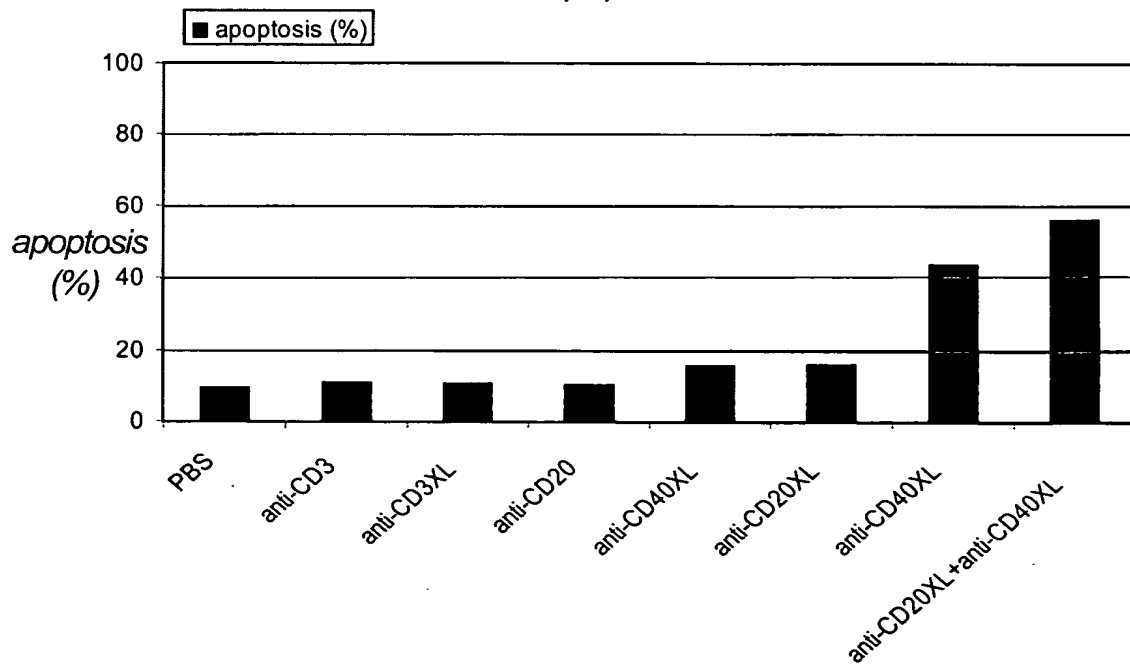


FIG.6B

Effect of Simultaneous ligation of CD20 and CD40 on CD95 and apoptosis.



HindIII NcoI 2H7 V₁ Leader Peptide →

M D F Q V Q I F S F L L I S A S

1 **AAGCTT**GCCG CC ATGGATT TCAAGTCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V_L →

61 V I I A R G Q I V L S Q S P A I L S A S
GTCATAATTG CCAGAGGACA AATTGTTC TCACAGTCTC CAGCAATCCT GTCTGCATCT

121 P G E K V T M T C R A S S S V S Y M H W
CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI

181 Y Q Q K P G S S P K P W I Y A P S N L A
TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCATC CAACCTGGCT

241 S G V P A R F S G S G S G T S Y S L T I
TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

301 S R V E A E D A A T Y Y C Q Q W S F N P
AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly₄Ser)₃ Linker →

361 P T F G A G T K L E L K G G G G S G G G
CCCACGTTTC GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H →

421 G S G G G G S S Q A Y L Q Q S G A E L V
GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

481 R P G A S V K M S C K A S G Y T F T S Y
AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

541 N M H W V K Q T P R Q G L E W I G A I Y
AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

601 P G N G D T S Y N Q K F K G K A T L T V
CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

661 D K S S S T A Y M Q L S S L T S E D S A
GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

721 V Y F C A R V V Y Y S N S Y W Y F D V W
GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

FIG.7B

human CD154/amino acid 48→

site	Bcl/Bam hybrid
781	G T G T T V T V S D P R R L D K I E D E GGCACAGGGA CCACGGTCAC CGTCTCT GTAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA
841	R N L H E D F V F M K T I Q R C N T G E AGGAATCTTC ATGAAGATTT TGTATTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA
901	R S L S L L N C E E I K S Q F E G F V K AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG
	BclI
961	D I M L N K E E T K K E N S F E M Q K G GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT
	BclI
	~~~~~
1021	D Q N P Q I A A H V I S E A S S K T T S GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT
1081	V L Q W A E K G Y Y T M S N N L V T L E GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA
1141	N G K Q L T V K R Q G L Y Y I Y A Q V T AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC
	HindIII
	~~~~~
1201	F C S N R E A S S Q A P F I A S L C L K TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG
1261	S P G R F E R I L L R A A N T H S S A K TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA
1321	P C G Q Q S I H L G G V F E L Q P G A S CCTTGCGGGC AACAATCCAT TCACTTGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG
	NcoI
	~~~~~
1381	V F V N V T D P S Q V S H G T G F T S F GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT
	XhoI XbaI
	~~~~~
1441	G L L K L E * * S R GGCTTACTCA AACTCGAGTG ATAATCTAGA

FIG.7C

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

```

HindIII      NcoI
-----
-----2H7 VL Leader Peptide→
      M D F Q V Q I F S F L L I S A S
1 AAGCTTGCCG CC ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                2H7 VL →
      V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                BamHI
                                -----
      Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

      S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly4Ser)3 Linker →
      P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                2H7 VH →
      G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

      P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

```

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FIG.7D

human CD154/amino acid 108 →

```

      BclI                      Bcl/Bam hybrid site
      G T G T T V T V S D P E N S F E M Q K G
781  GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

      BclI
      -----
      D Q N P Q I A A H V I S E A S S K T T S
841  GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAATCT

      V L Q W A E K G Y Y T M S N N L V T L E
901  GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

      N G K Q L T V K R Q G L Y Y I Y A Q V T
961  AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

      HindIII
      -----
      F C S N R E A S S Q A P F I A S L C L K
1021 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

      S P G R F E R I L L R A A N T H S S A K
1081 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

      P C G Q Q S I H L G G V F E L Q P G A S
1141 CCTTGCGGGC AACAAATCCAT TCACTTGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

      NcoI
      -----
      V F V N V T D P S Q V S H G T G F T S F
1201 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

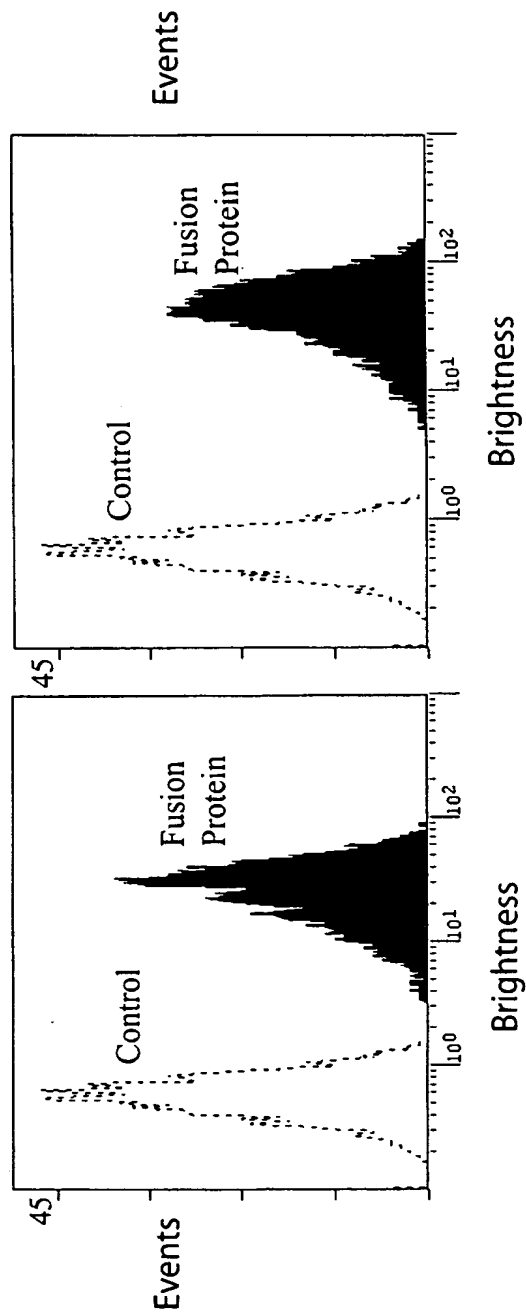
      XhoI                      XbaI
      -----
      G L L K L E * * S R
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA
```

FIG.8

Simultaneous Binding of 2H7scFv-CD154
Fusion Proteins to CD20 and CD40

2H7scFv-CD154
Construct S4

2H7scFv-CD154
Construct L2

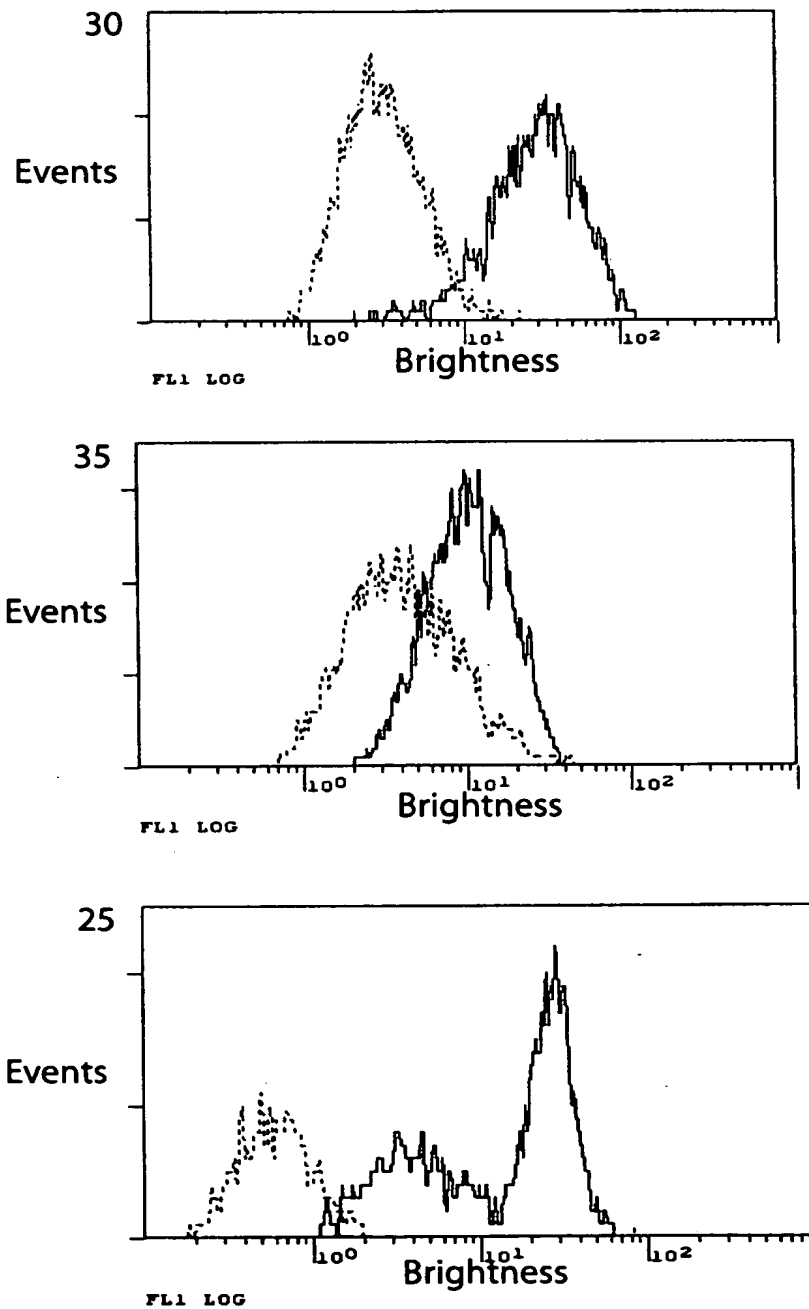


CD20 CHO cell targets + (control or fusion protein)
+ Biotin-CD40Ig + PE-SA

FIG.9

Induction of Apoptosis Measured by Binding of Annexin V after incubation with 2H7scFv-CD154

(2) :G0019085.LMD FL1 LOG R1



.....control supernatant ___ 2H7scFv-CD154 supernatant

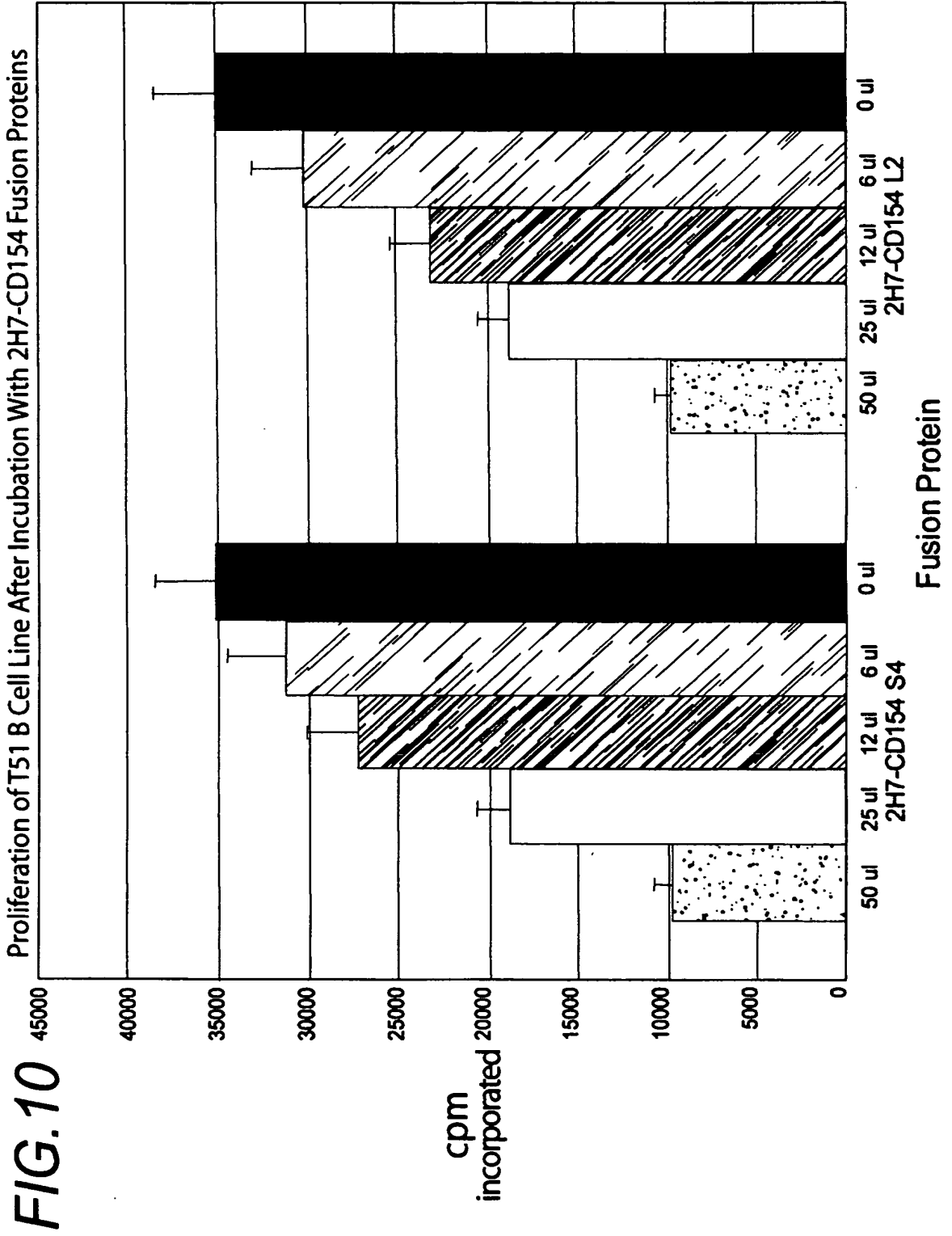


FIG. 11

2H7 scFv (SSS-S)H WCH2 WCH3
 OR 2H7 scFv (SSS-S)H P238SCH2 WCH3

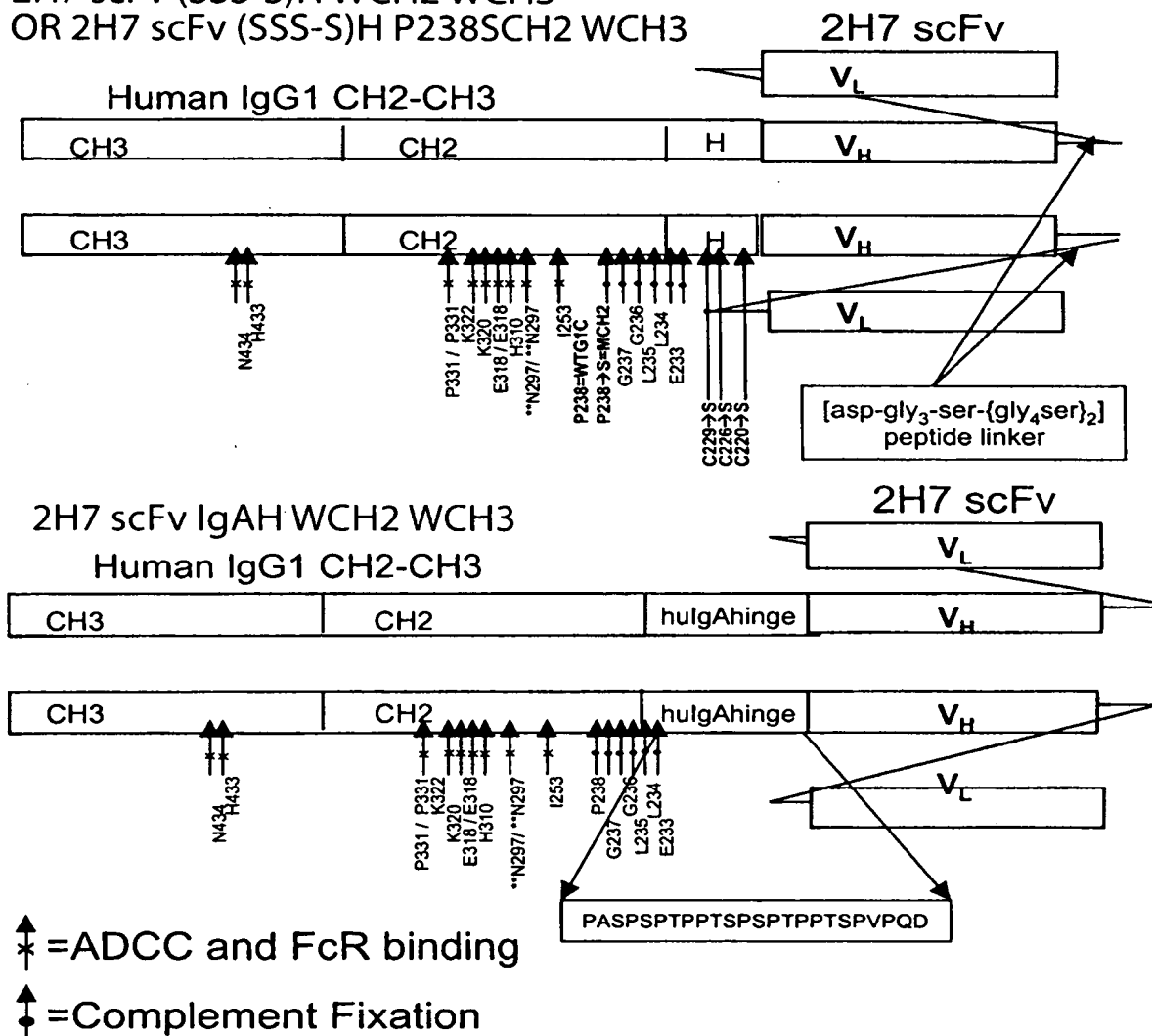


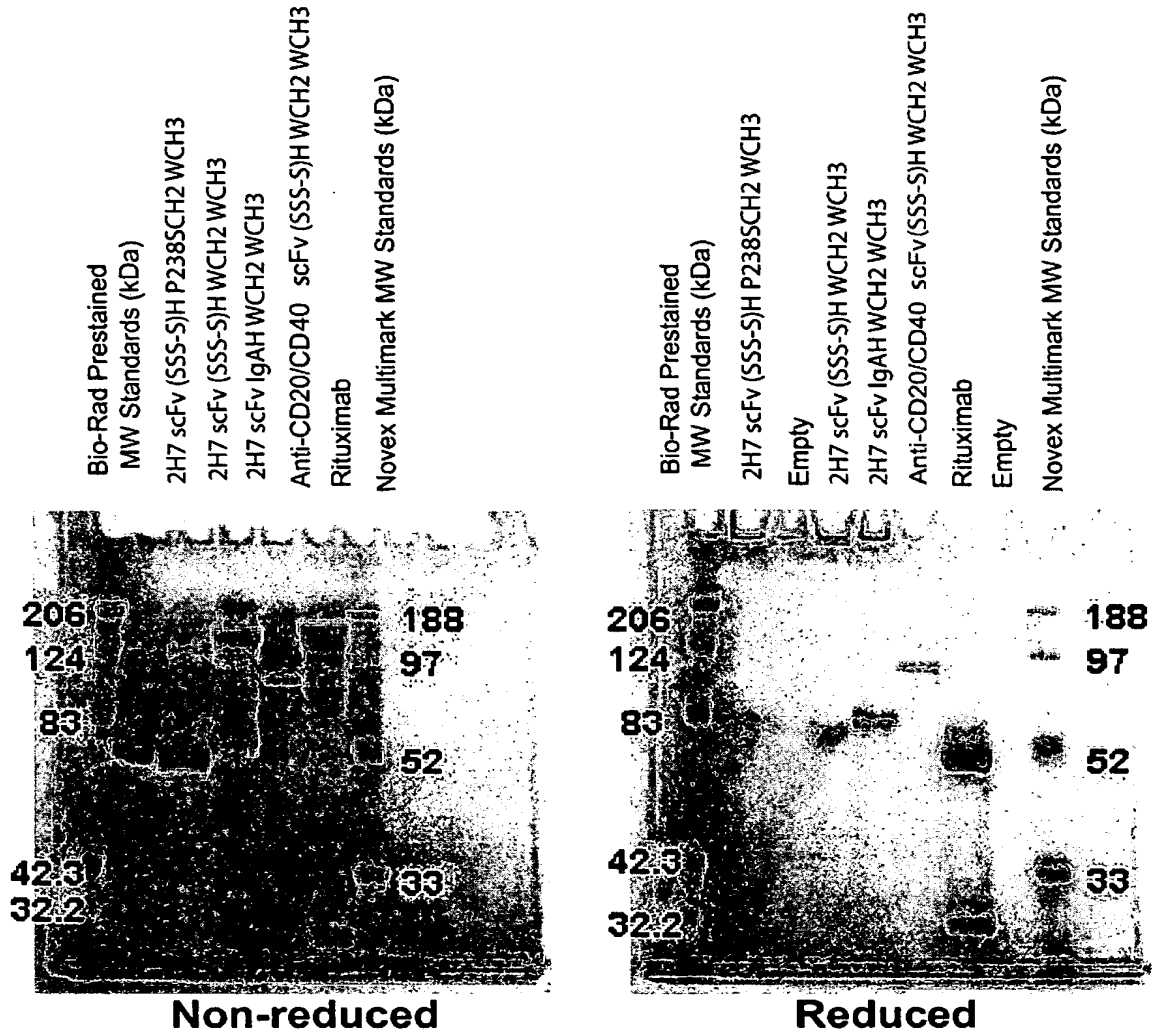
FIG. 12

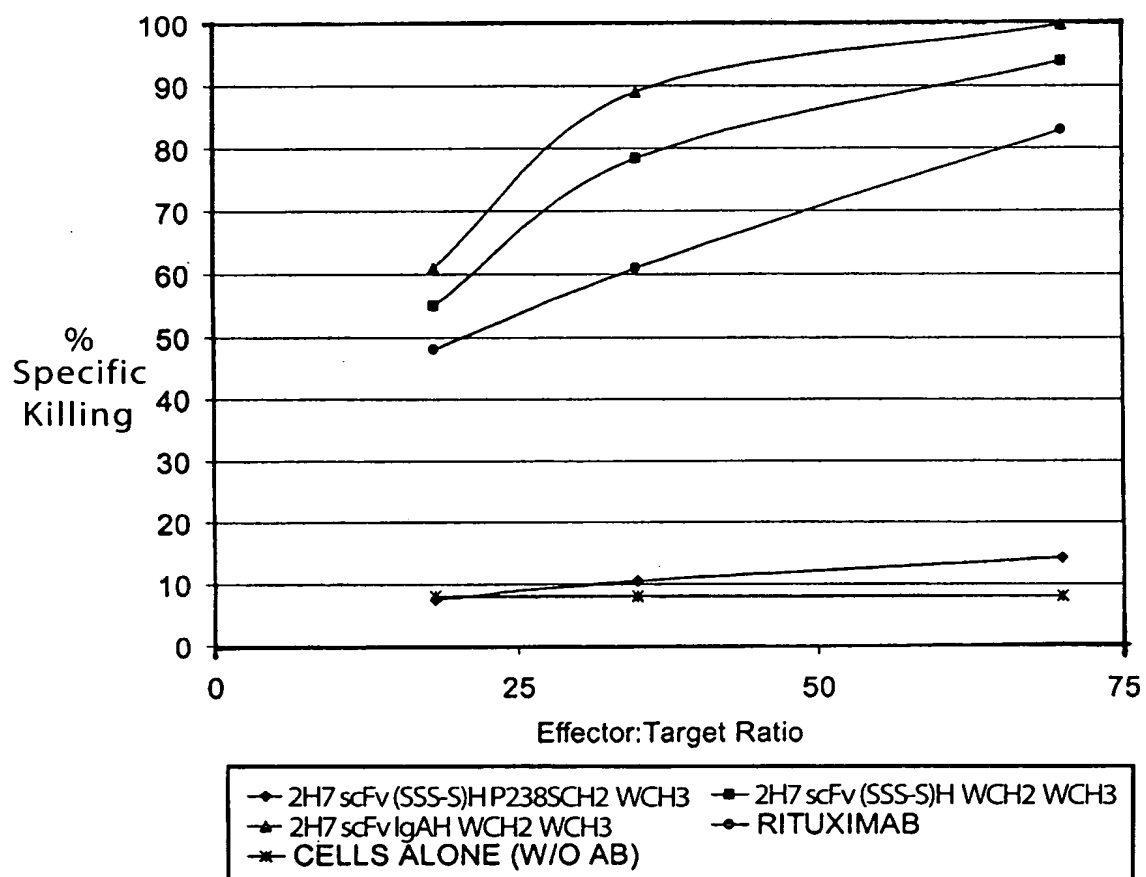
FIG.13**ADCC Activity of 2H7 scFv Constructs**

FIG. 14

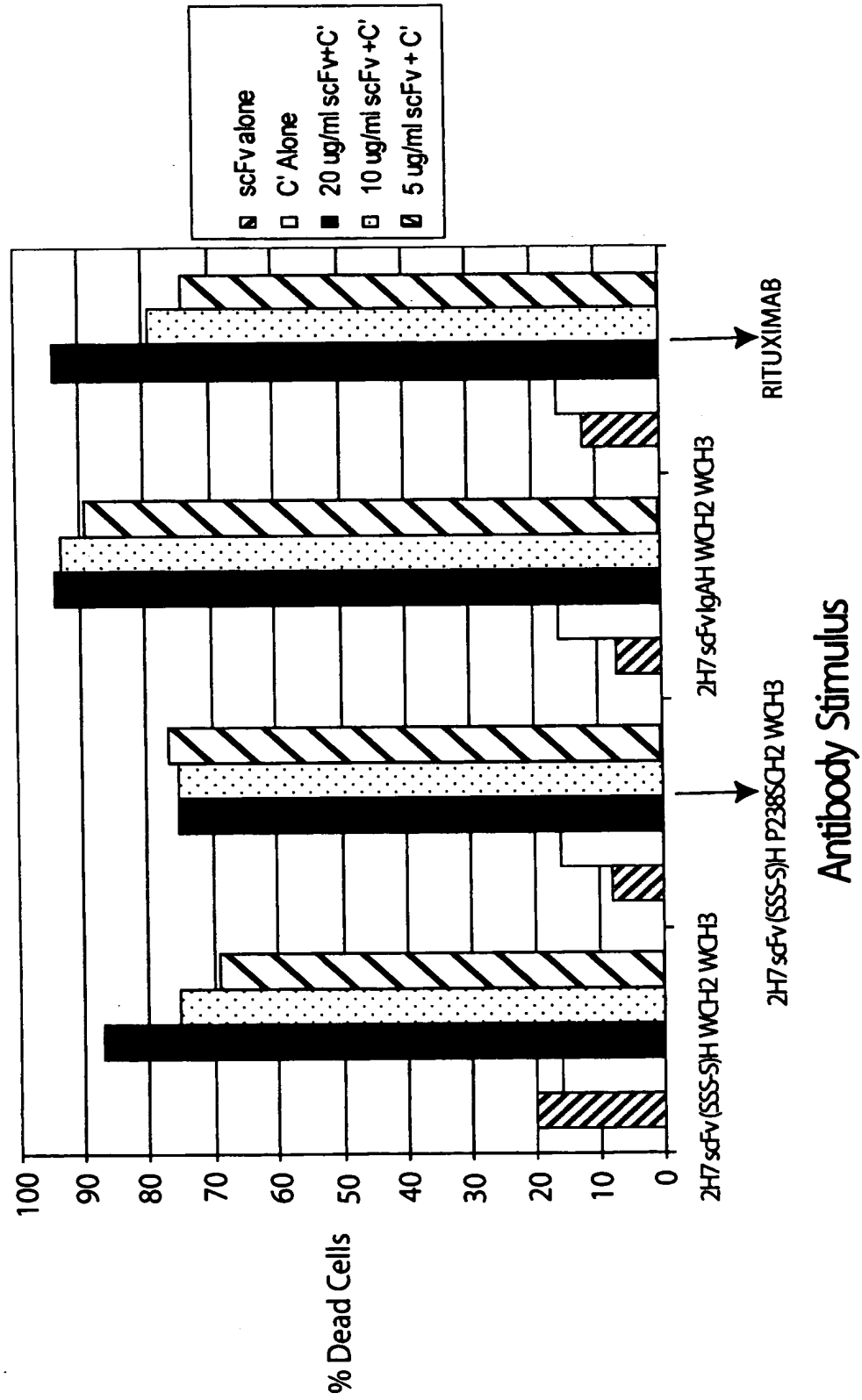
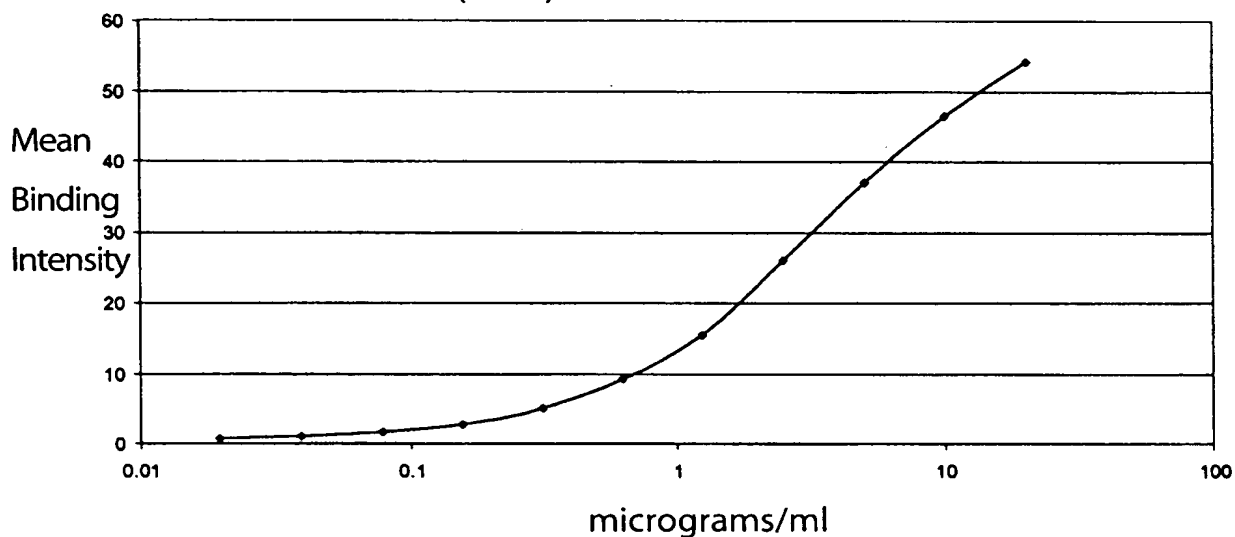


FIG. 15

2H7 scFv (SSS-S)H WCH2 WCH3 In Vivo Half Life

2H7 scFv (SSS-S)H WCH2 WCH3 Standard Curve



Macaque A99314

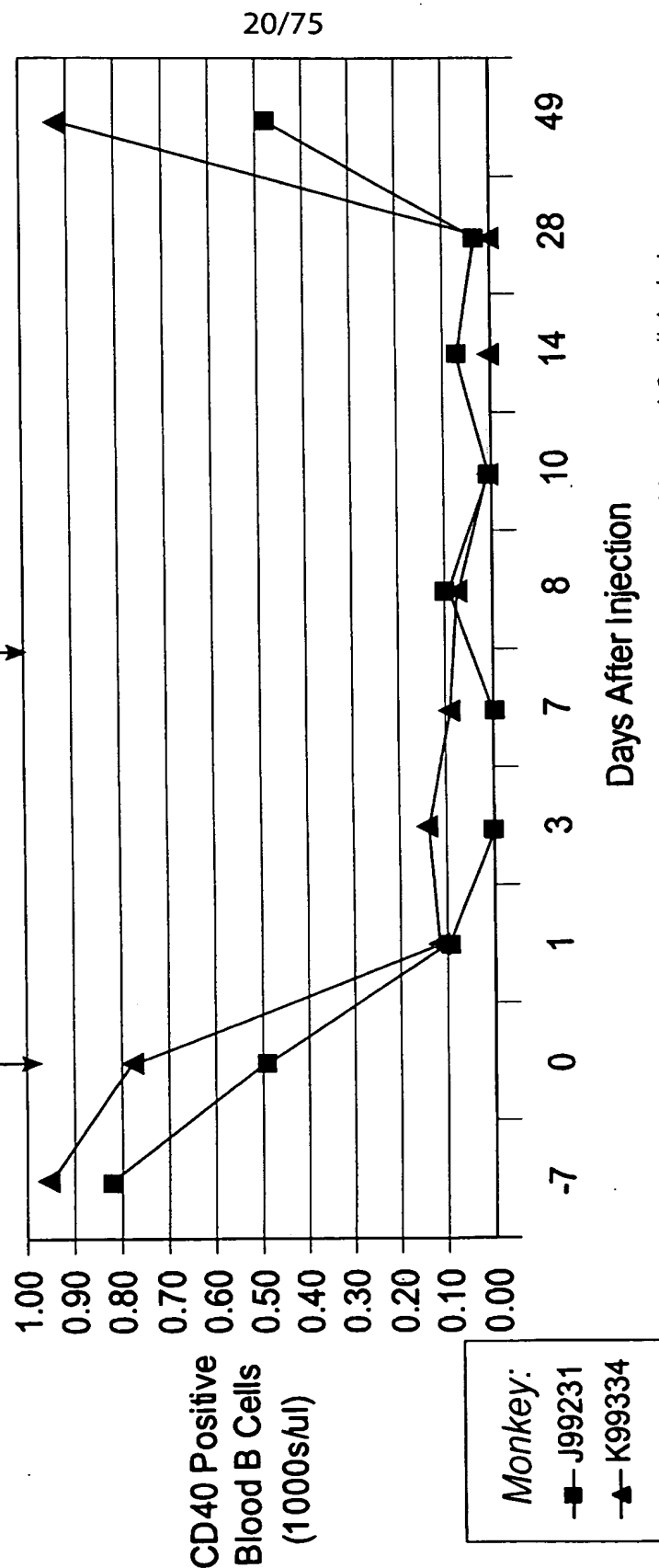
	Day	Binding intensity At 1:50	estimated concentration (µg/ml)
	-7	0.213	<0.1
Injection #1	0	0.227	<0.1
	1	7.79	25.1
	3	5.51	15.6
Injection #2	7	3.37	9.4
	8	11.33	41.7
	10	5.45	15.4
	14	0.27	<0.1

Macaque F98081

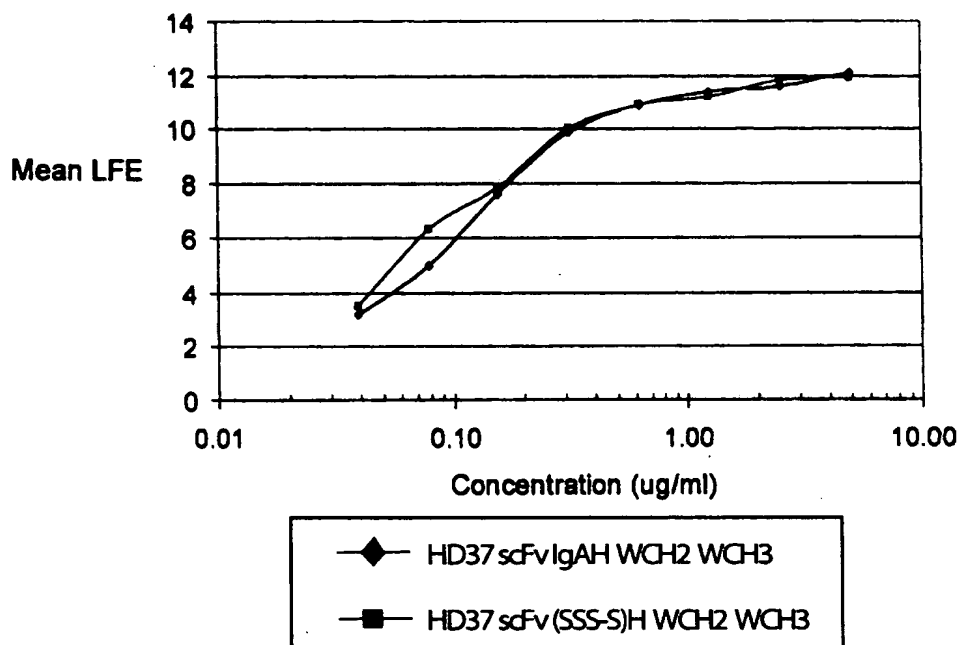
	Day	Binding intensity At 1:50	estimated concentration (µg/ml)
	-7	0.208	<0.1
Injection #1	0	0.219	<0.1
	1	6.73	21.9
	3	6.14	19.3
	7	3.04	8.7
Injection #2	8	9.83	33.8
	10	4.77	14.4
	14	0.231	<0.1

FIG. 16

B Cell Depletion in macaques mediated by
2H7 scFv (SSS-S)H WCH2 WCH3

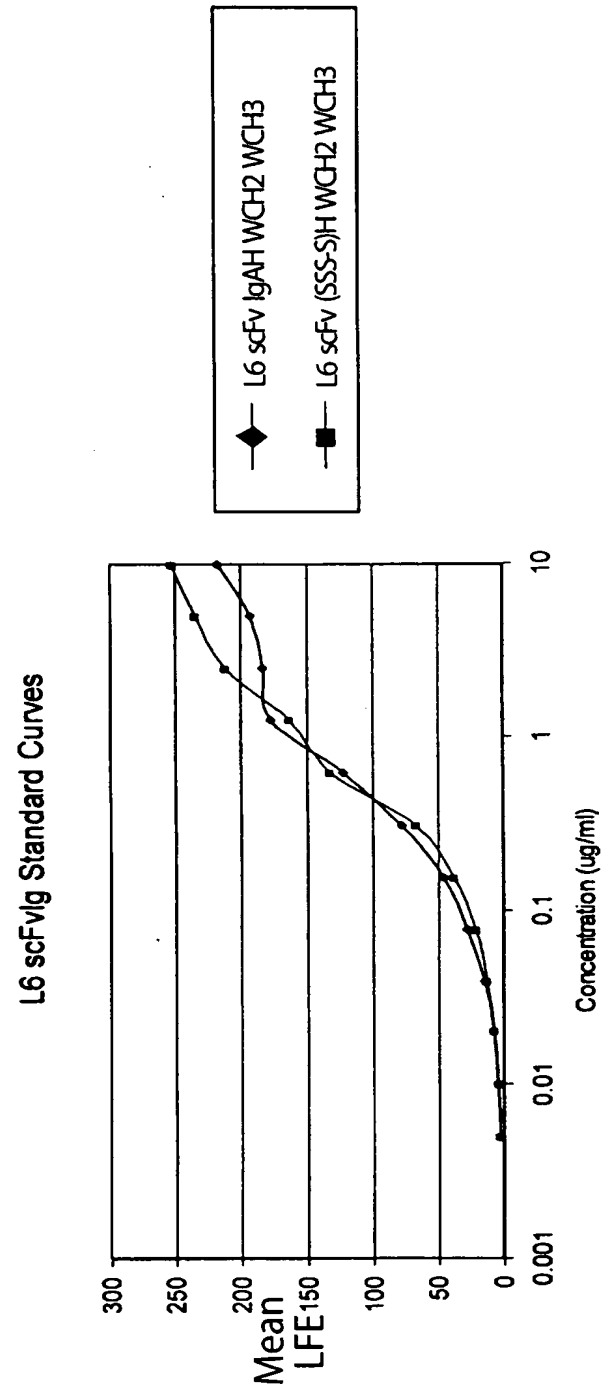


- 2H7 scFv (SSS-S)H WCH2 WCH3 Injections of 6mg/kg yields 3 week B-cell depletion
- 3-4 day half-life *in vivo*
- CD20 saturation in lymph node B-cells at d14
- No first dose effects
- No anti-chimeric antibody development

FIG.17**Production Levels of HD37 scFv Constructs by CHO Cell Lines****Standard Curve of HD37 scFvlg Constructs
Binding to B Cells**

<u>Clone/isolate</u>	<u>Mean LFE at 1:100</u>	<u>Estimated Concentration</u>
HD37 scFvlgAH WCH2 WCH3	11.2	> 60 ug/ml
1B2	10.4	>50 ug/ml
6C5	10.5	>50 ug/ml
4B1	8.6	>40 ug/ml
HD37 scFv(SSS-S)H WCH2 WCH3	10.9	> 50 ug/ml
2G8	10.6	> 50 ug/ml
3F3	8.3	>40 ug/ml
3D9	11.1	> 60 ug/ml

FIG.18 Production of L6 scFvlg Constructs by CHO Cells



<u>Construct</u>	<u>Mean LFE 1:20</u>	<u>Estimated Concentration</u>
L6 scFv IgAH WCH2 WCH3 unamplified CHO sup	51.1	6.25 ug/ml
L6 scFv(SSS-S)H WCH2 WCH3 unamplified CHO sup	23.0	3.2 ug/ml

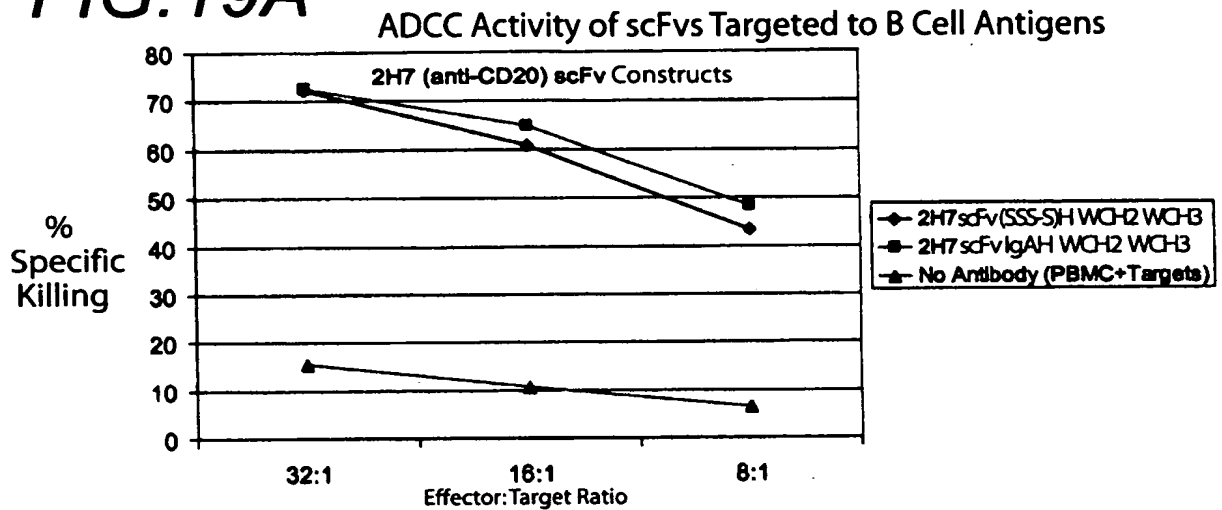
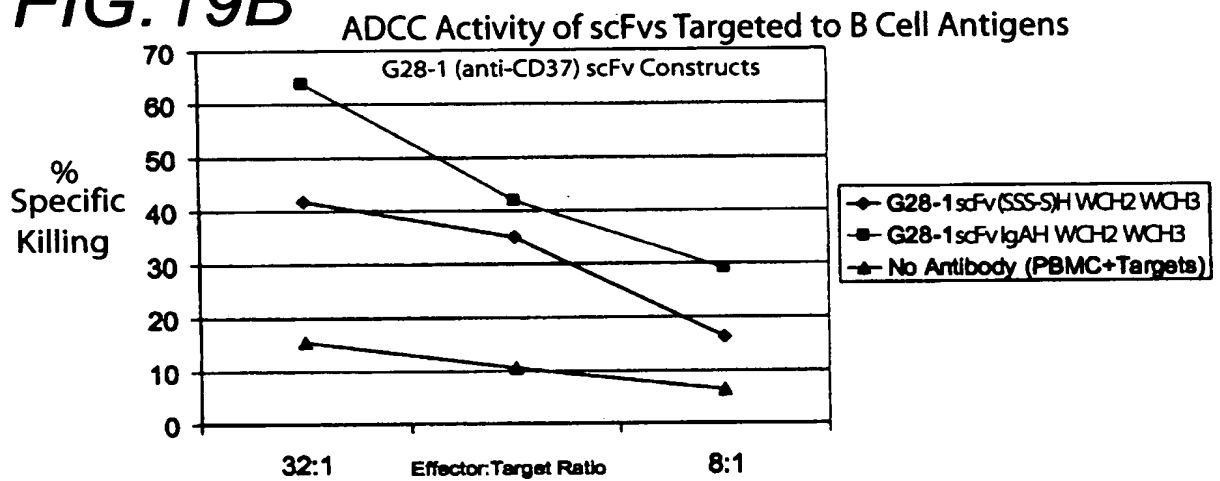
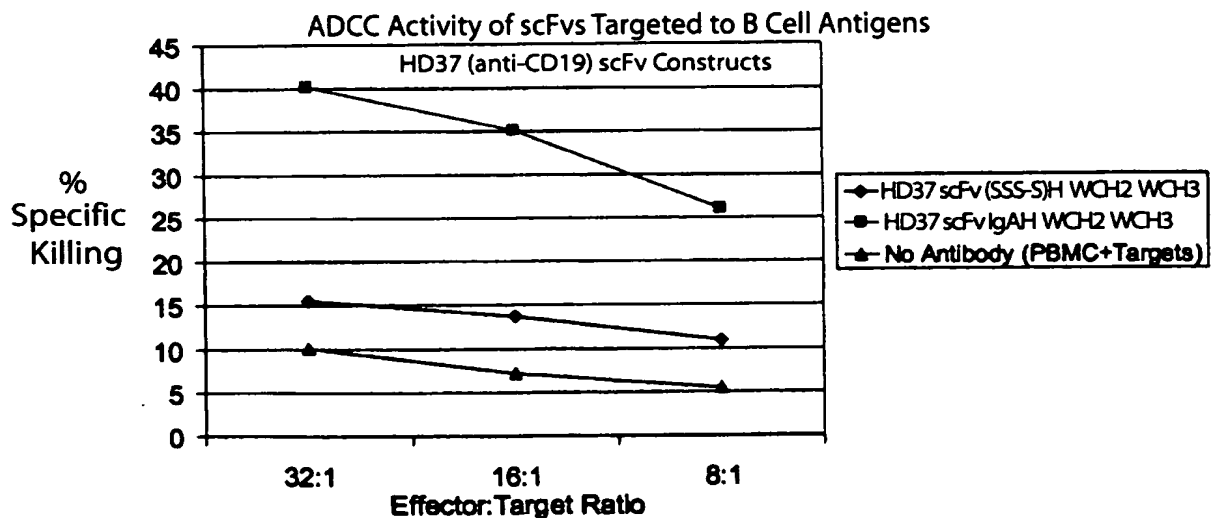
FIG. 19A**FIG. 19B****FIG. 19C**

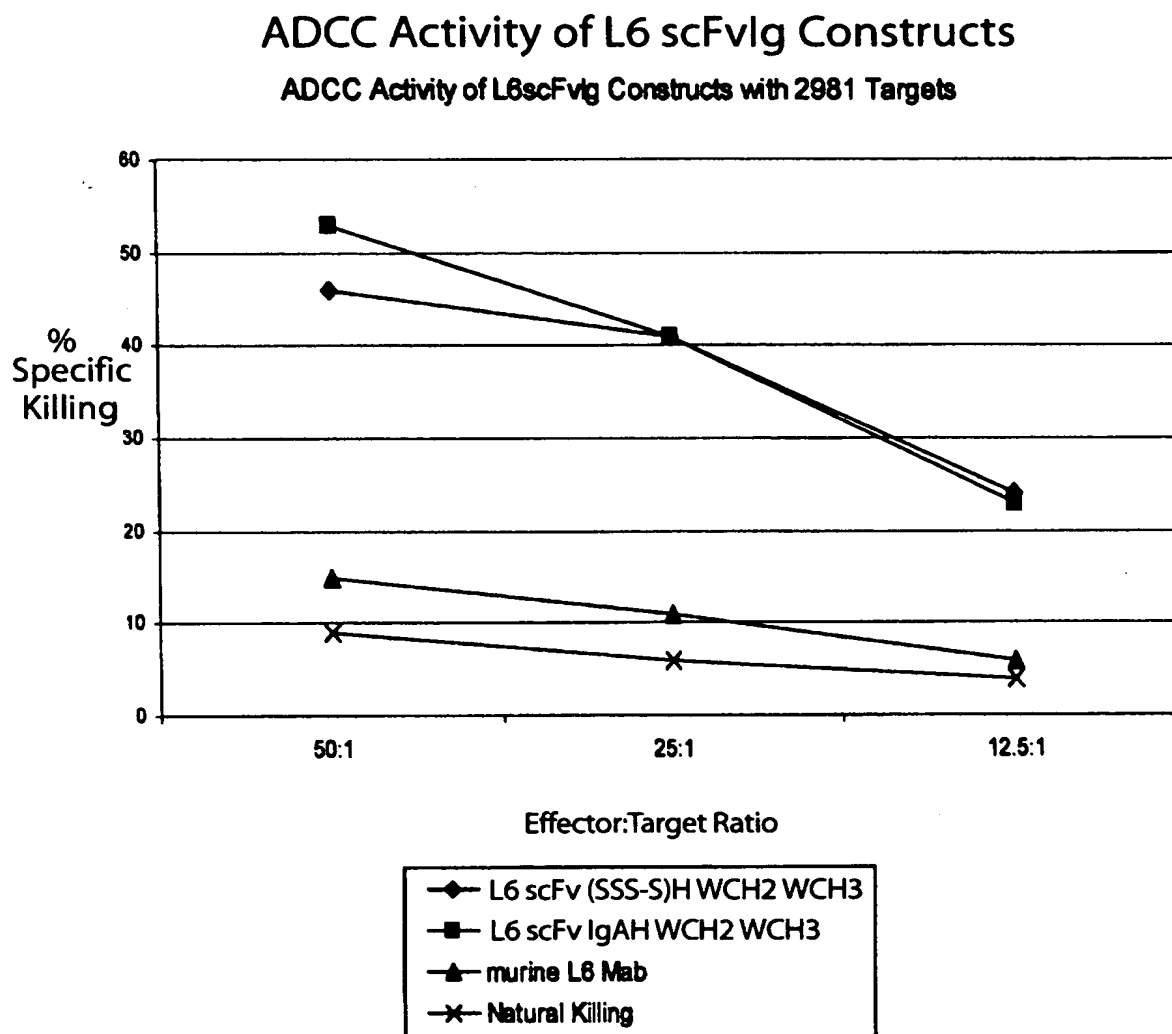
FIG.20

FIG.21

SDS-PAGE Analysis of L6 and 2H7
scFvlg Constructs

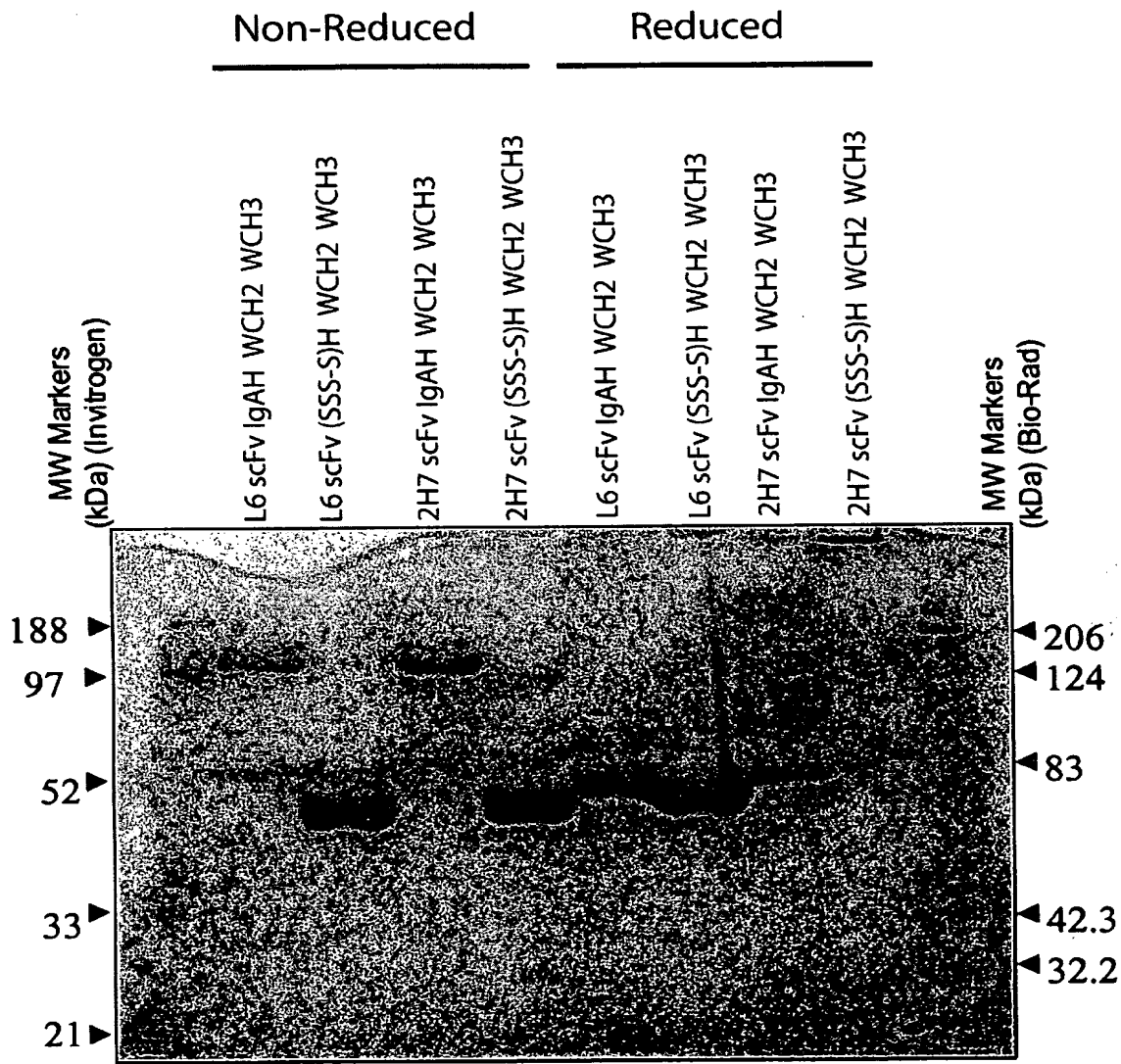


FIG.22

SDS-PAGE Analysis of G28-1 and HD37 scFvlg Constructs

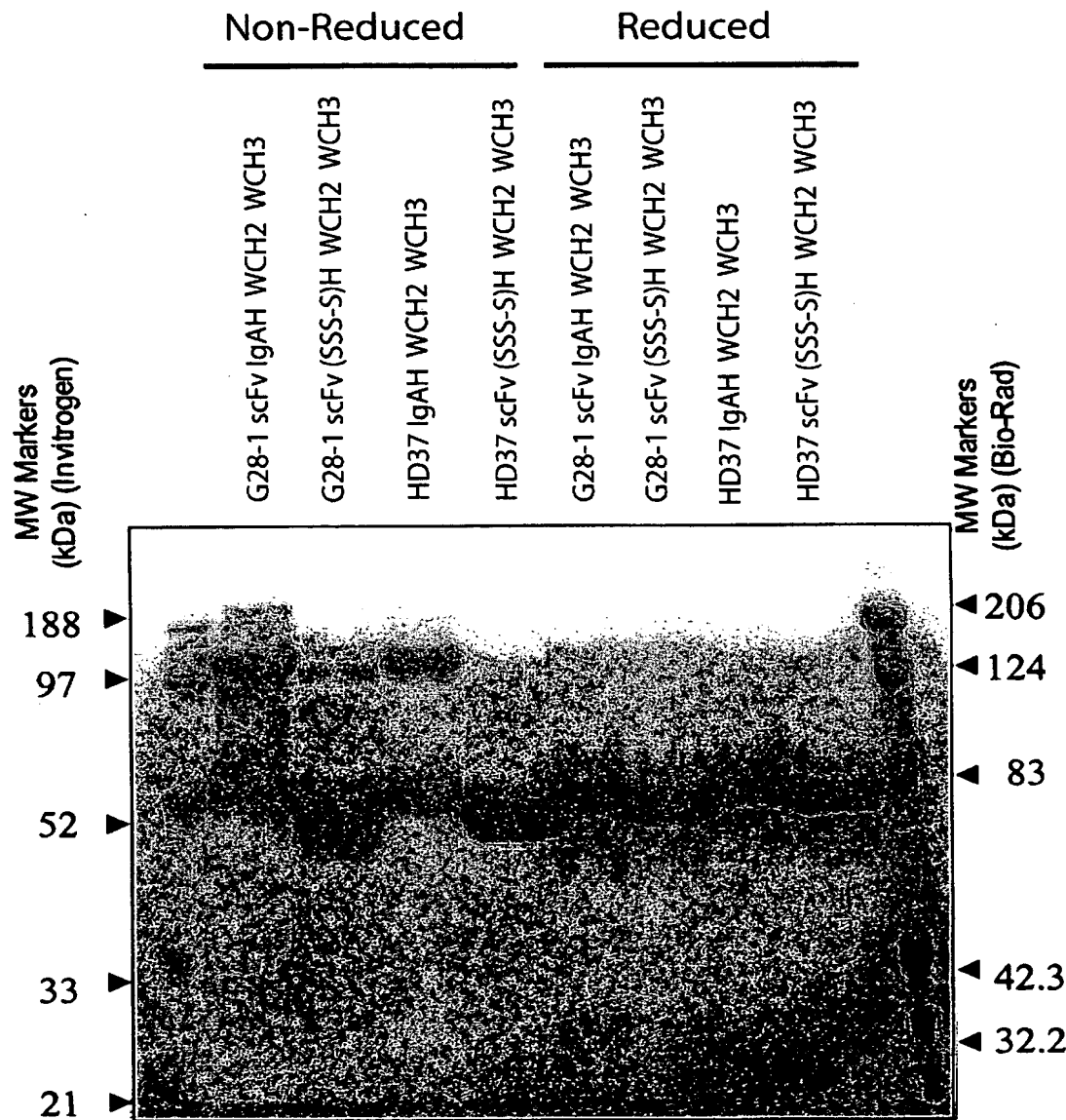


FIG.23

Sequence alignment of human and Llama Fc regins.

	HINGE	CH2 →
Human IgG1:	DQEPKSCDKT-----HTCPPQ	PAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
Llama IgG2:	DQEPKTPKPQPQPQPNPTTESKCPKQ	PAPELLGGPSVFLFPPKPKDVLISIGRPEVTCVVVDVGQEDPEVSFNWYIDG
Llama IgG1:	--EPHGG-----CTCPQC	PAPELPGGPSVFLFPPKPKDVLISIGRPEVTCVVVDVGKEDPEVNFNWIYIDG
Llama IgG3:	--AHHSEDPT-----SKCPKQ	PGPELLGGPTVFLFPPKAKDVLISITRKPEVTCVLTWVWVKKTLRSSSSWSVDD

VEVHNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
 TAEVRANTRPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIEKTISKAKGQTPREPQVYTLAPHREELAKDTVSVT
 VEVRTANTKPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIEKTISKAKGQTPREPQVYTLAPHREELAKDTVSVT
 TEVHTAETKPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIEKTISKAKGQTPREPQVYTLAPHREELAKDTVSVT

CLVKGYFSPDIAVEWESNGQPEN---NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
 CLVKGYFPDPINVEWQRNGQPESEGTYATTPQLDNDGTFLYSLXSVGKNTWQQGETFTCVVMHEALHNHYTQKSITQSSGK
 CLVKGYFPADINVEWQRNGQPESEGTYANTPPQLDNDGTFLYSLRSLVGKNTWQRGETLTGVVMHEALHNHYTQKSITQSSGK
 CLVKGFFPADINVEWQRNGQPESEGTYANTPPQLDNDGTFLYSLKSLVGKNTWQQGEVFTCVVMHEALHNHSTQKSITQSSGK

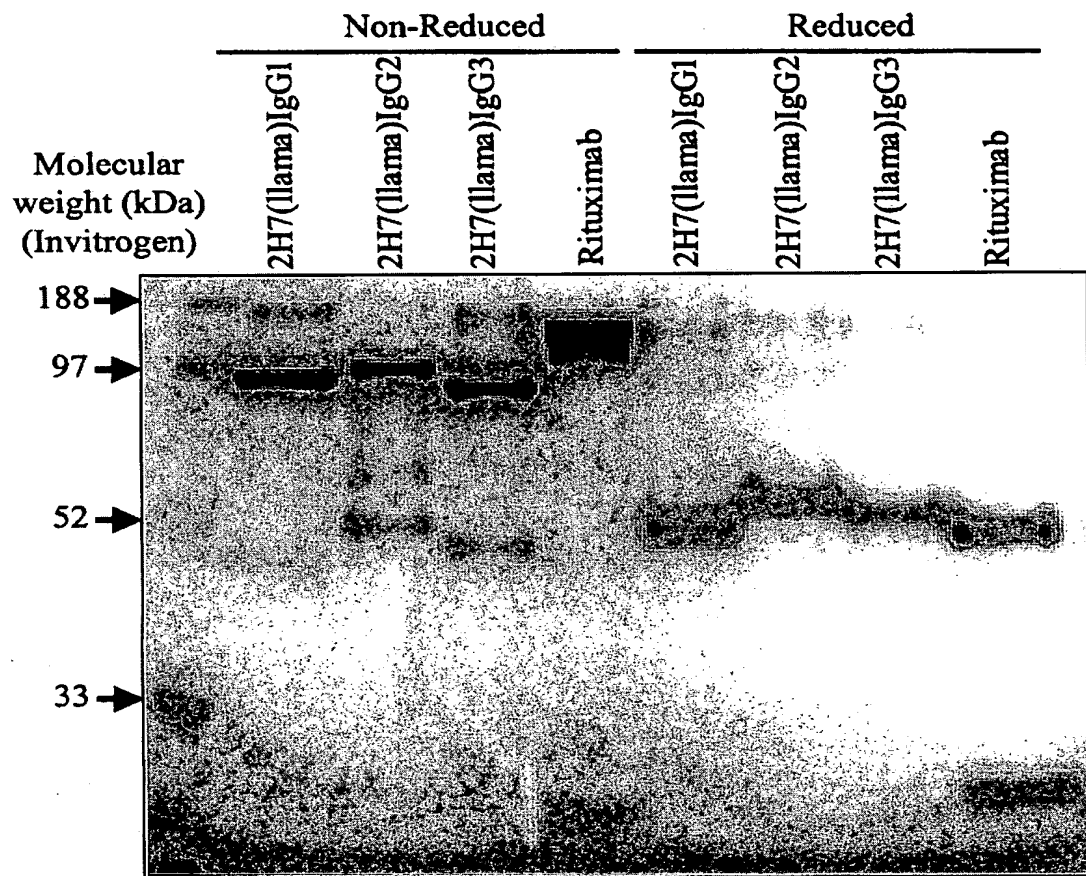
FIG.24

FIG.25

Llama Tails Binding Assay with CD20 CHO Cells

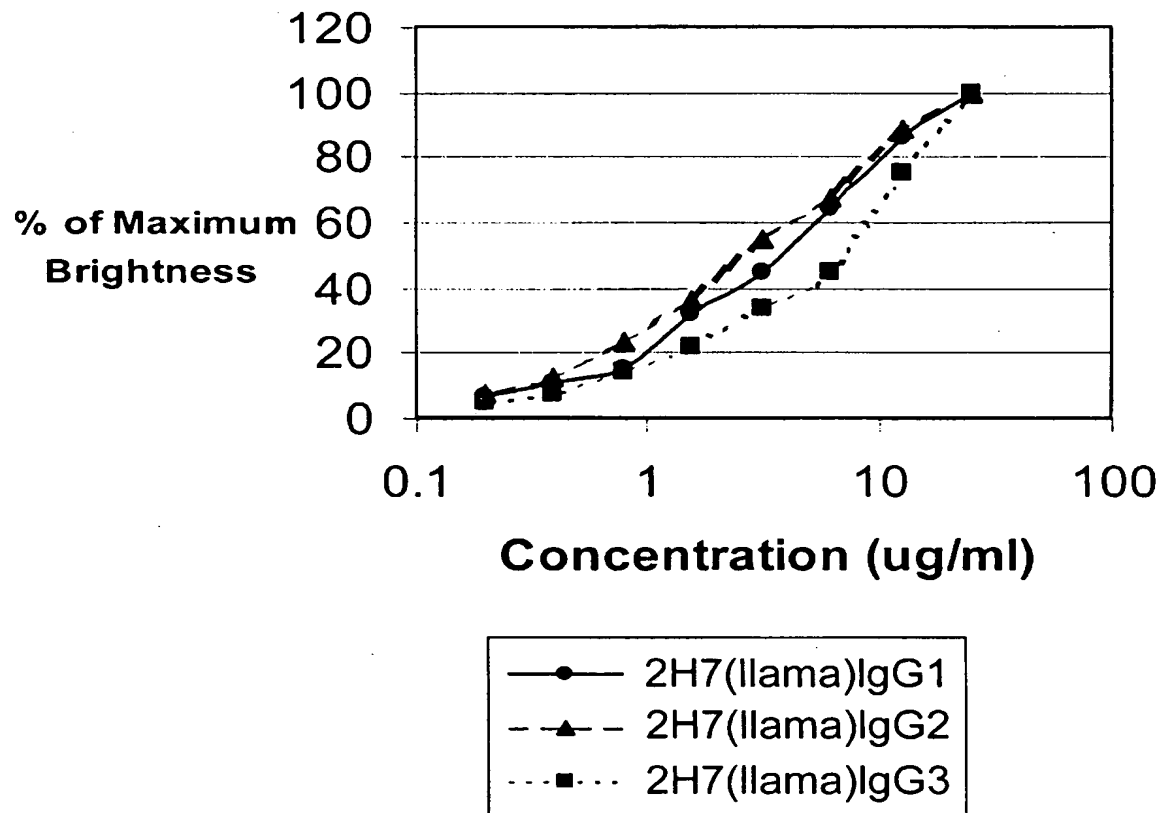


FIG.26

2H7 scFv Llama Constructs Complement Assay with BJAB Cells

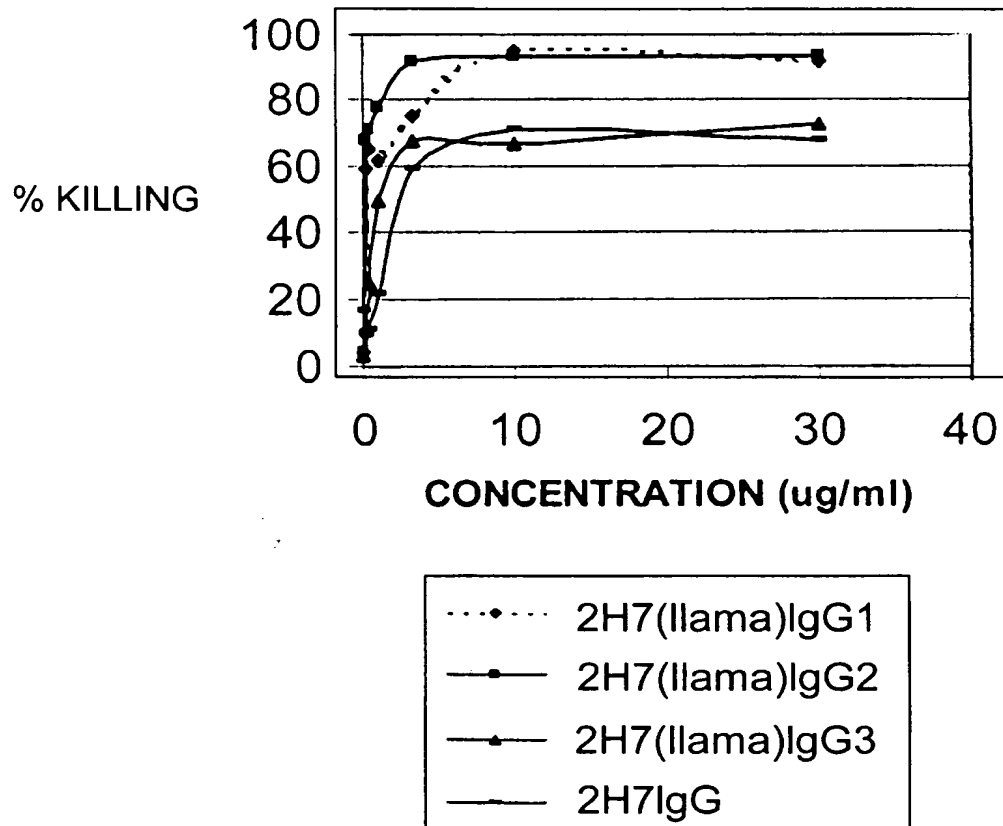


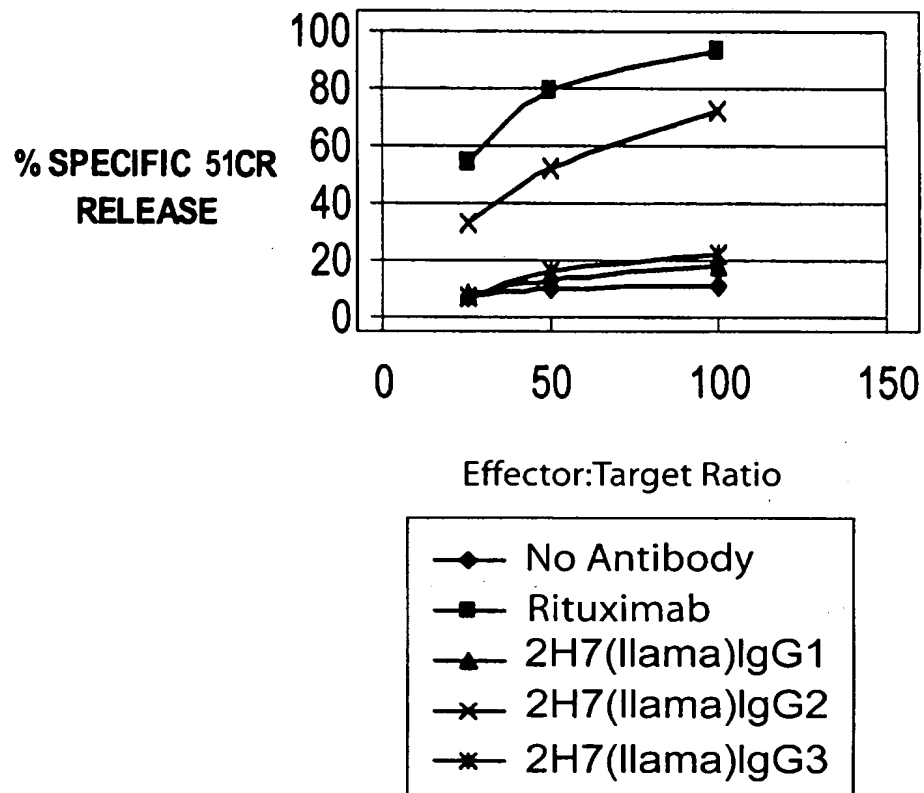
FIG.27**ADCC ASSAY WITH BJAB TARGETS
AND HUMAN PBMC EFFECTORS**

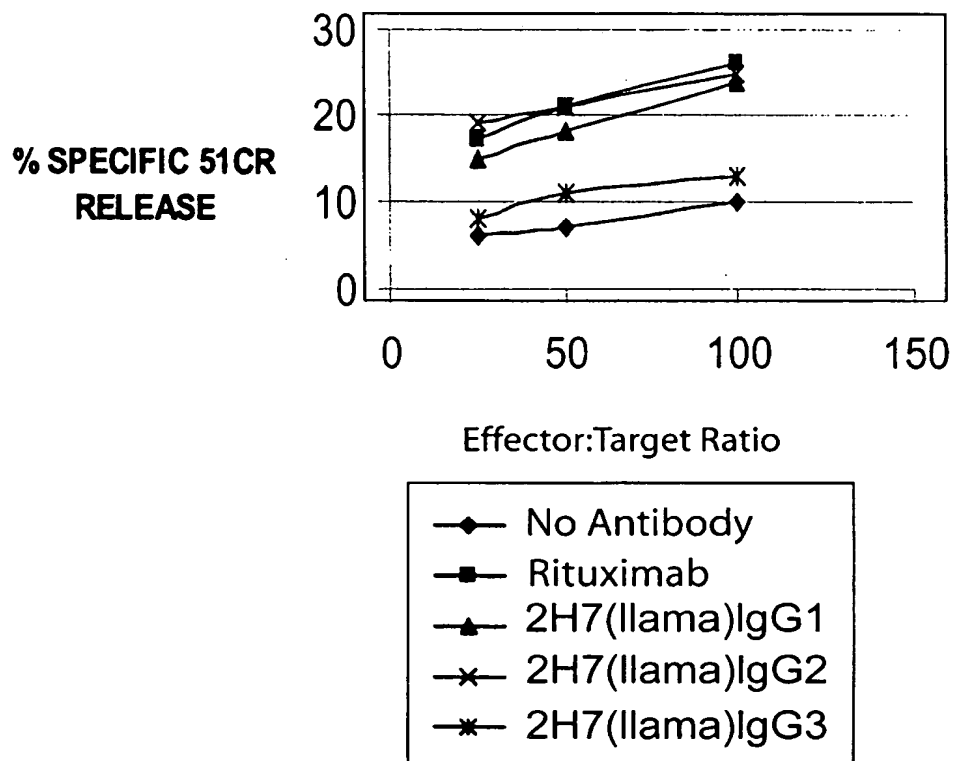
FIG.28**ADCC ASSAY WITH BJAB TARGETS
AND LLAMA PBMC EFFECTORS**

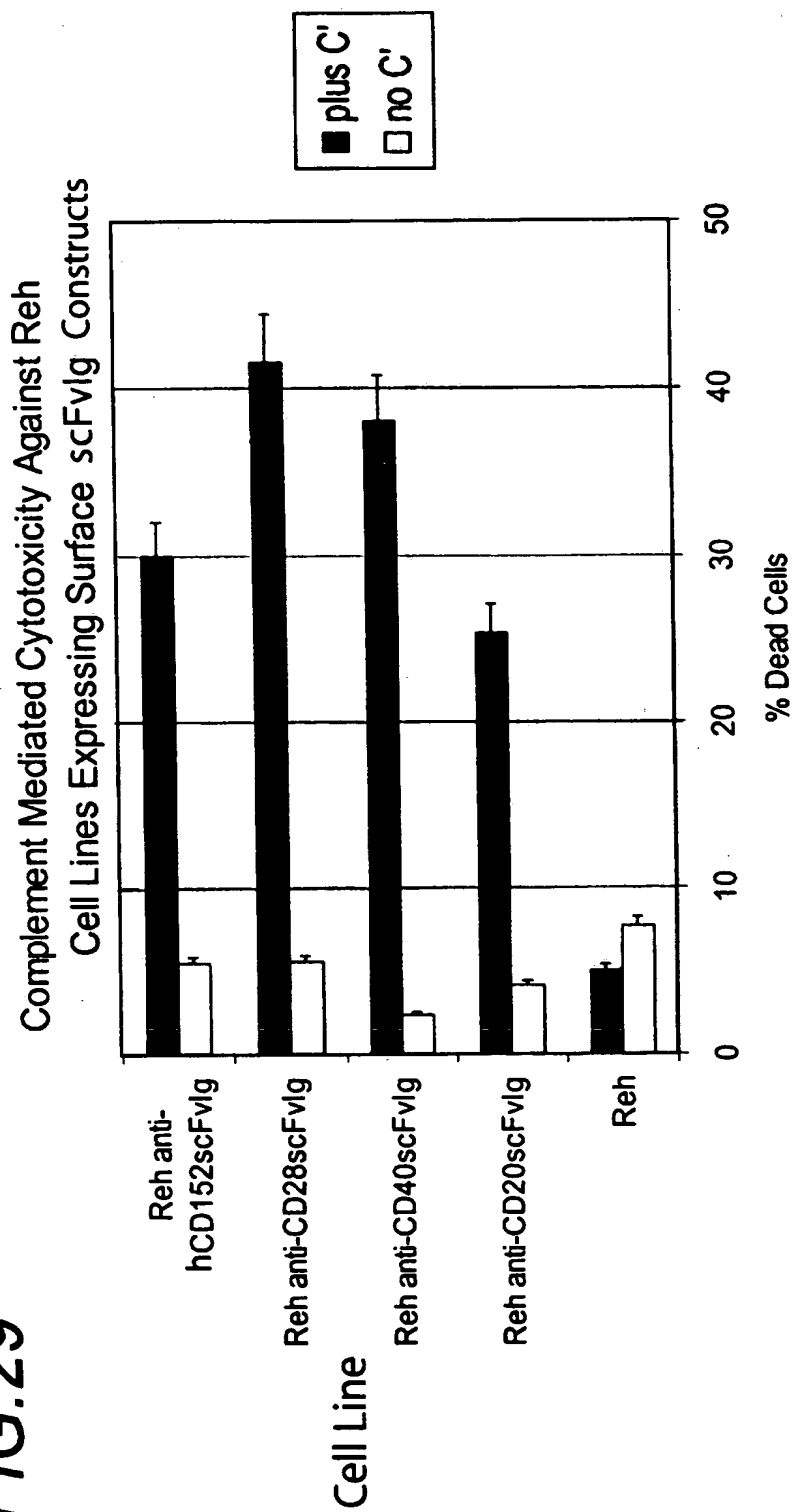
FIG. 29

FIG.30

ADCC Activity of Cell Surface Expressed ScFvlg Constructs

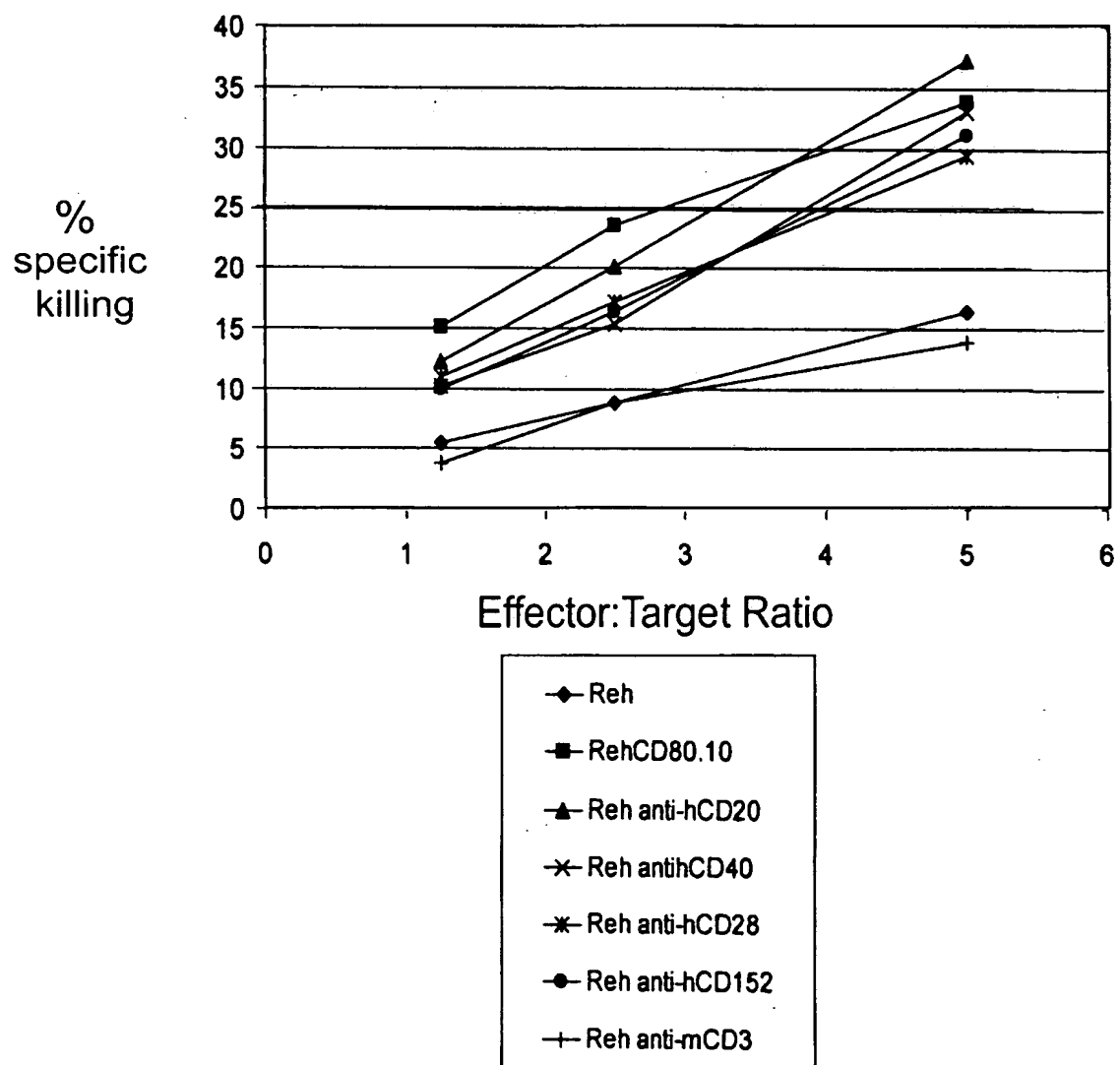


FIG.31 Ig Constructs and Nomenclature:

Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence
(CCC-P)WH WCH2 WCH3	IgG1 WT Hinge (CCC)	Wild Type CH2	Wild Type CH3
(SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
VHL115 (SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(SSC-P)H WCH2 WCH3	IgG1 Mutant Hinge (SSC)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(SCS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SCS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(CSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (CSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(SSS-S)H P238SCH2WCH3	IgG1 Mutant Hinge (SSS)	Mutant CH2 (IgG1) Pro→Ser 238	Wild type CH3 (IgG1)
IgAH WCH2 WCH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgAH IgA CH2CH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)
IgAH IgA CH2T4CH3	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) Missing 4 aa at COOH

FIG.32

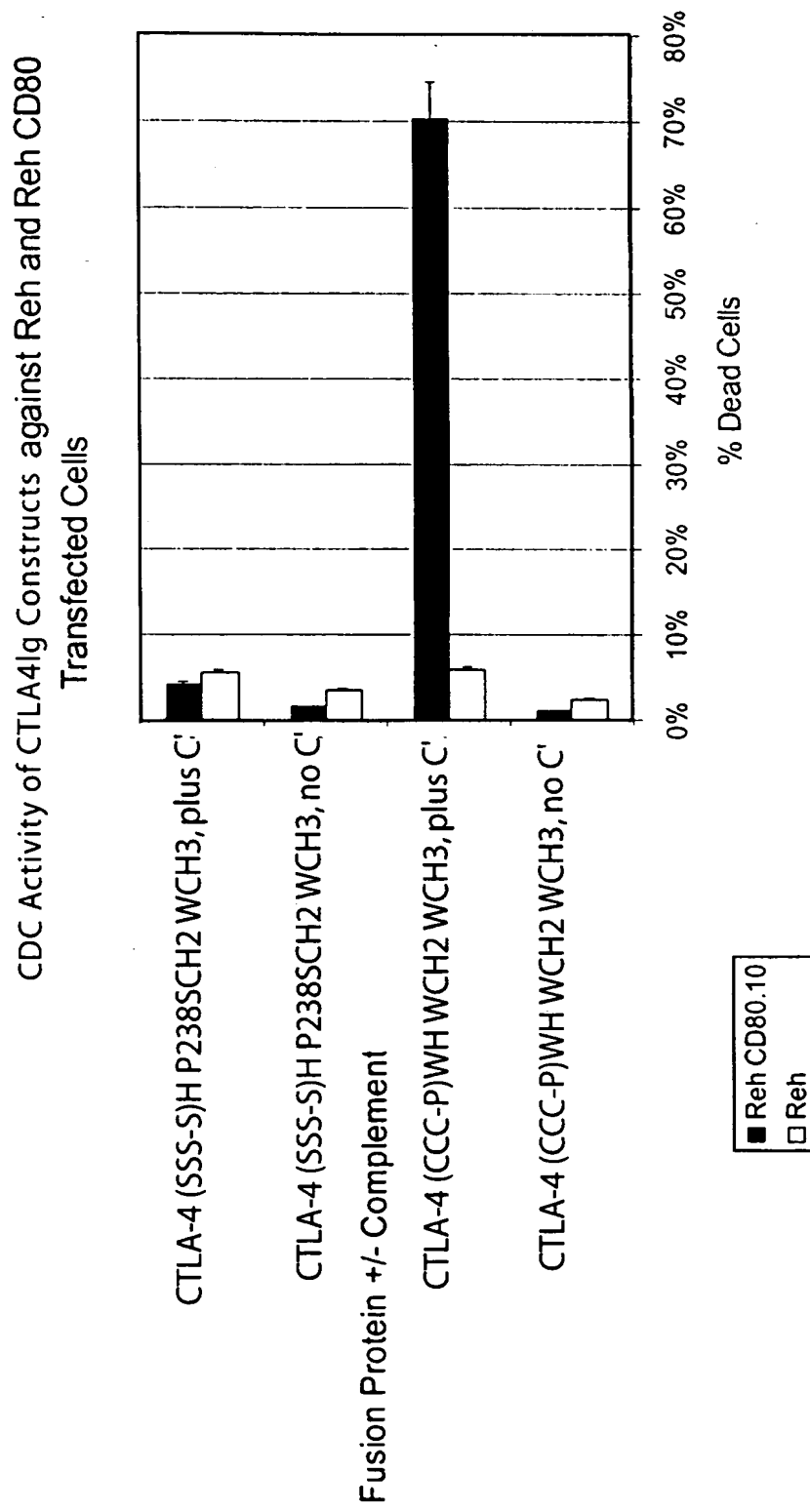


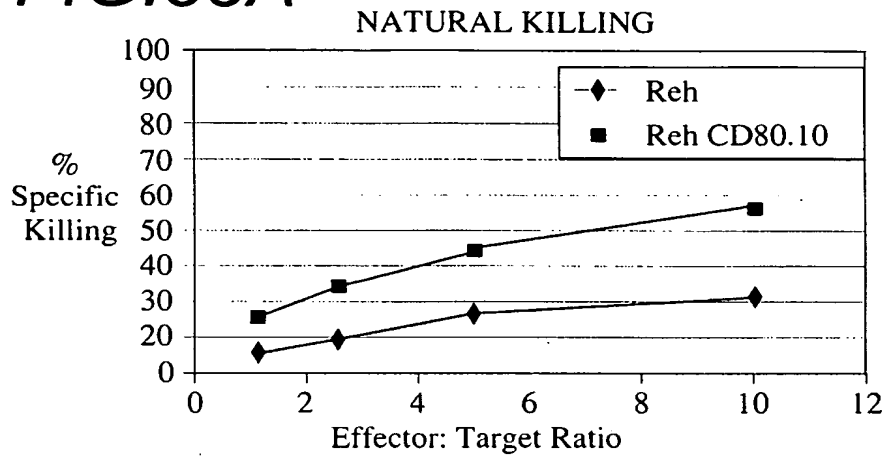
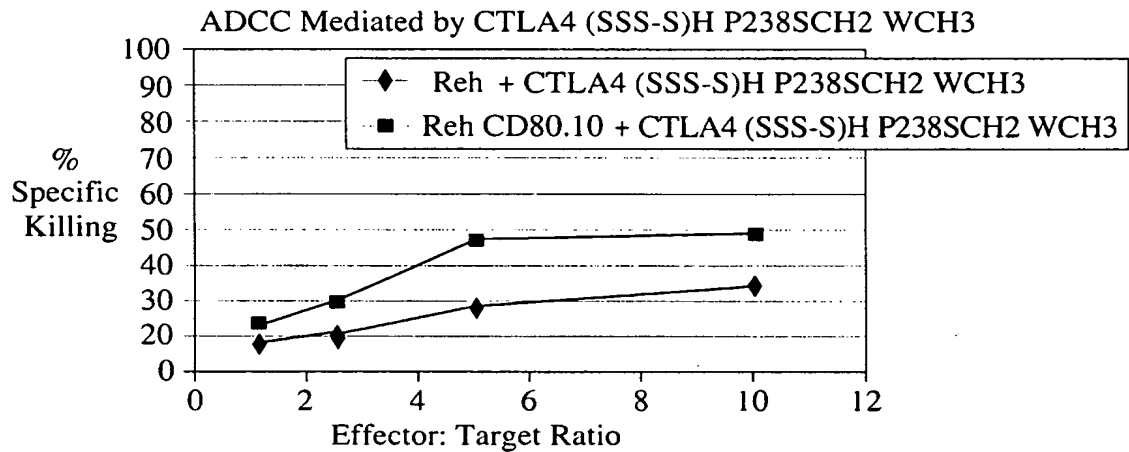
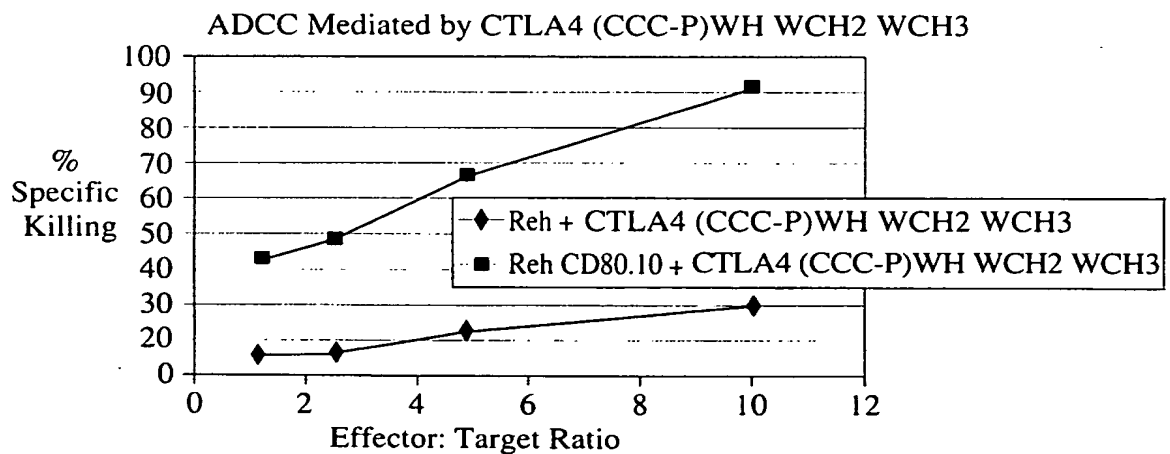
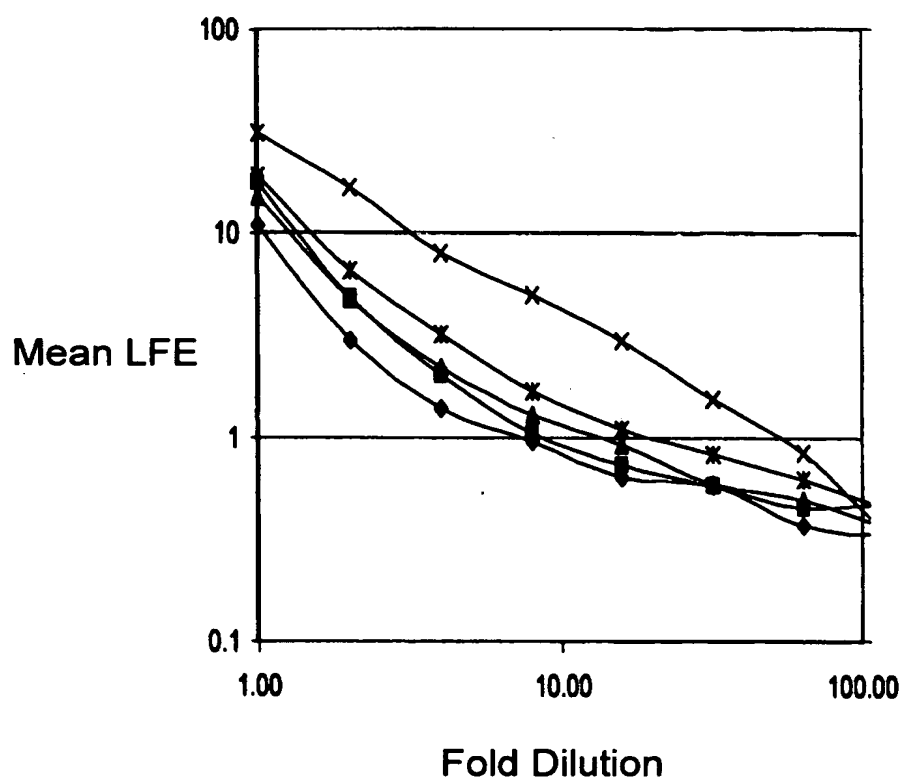
FIG.33A**FIG.33B****FIG.33C**

FIG.34**Binding of Constructs Alternative to CD2 CHO Cells**

- ◆ 2H7 scFv (CCC-P)WH WCH2 WCH3
- 2H7 scFv (CSS-S)H WCH2 WCH3
- ▲ 2H7 scFv (SCS-S)H WCH2 WCH3
- ✱ 2H7 scFv (SSC-P)H WCH2 WCH3
- ✱ 2H7 scFv VHL11S (SSS-S)H WCH2 WCH3

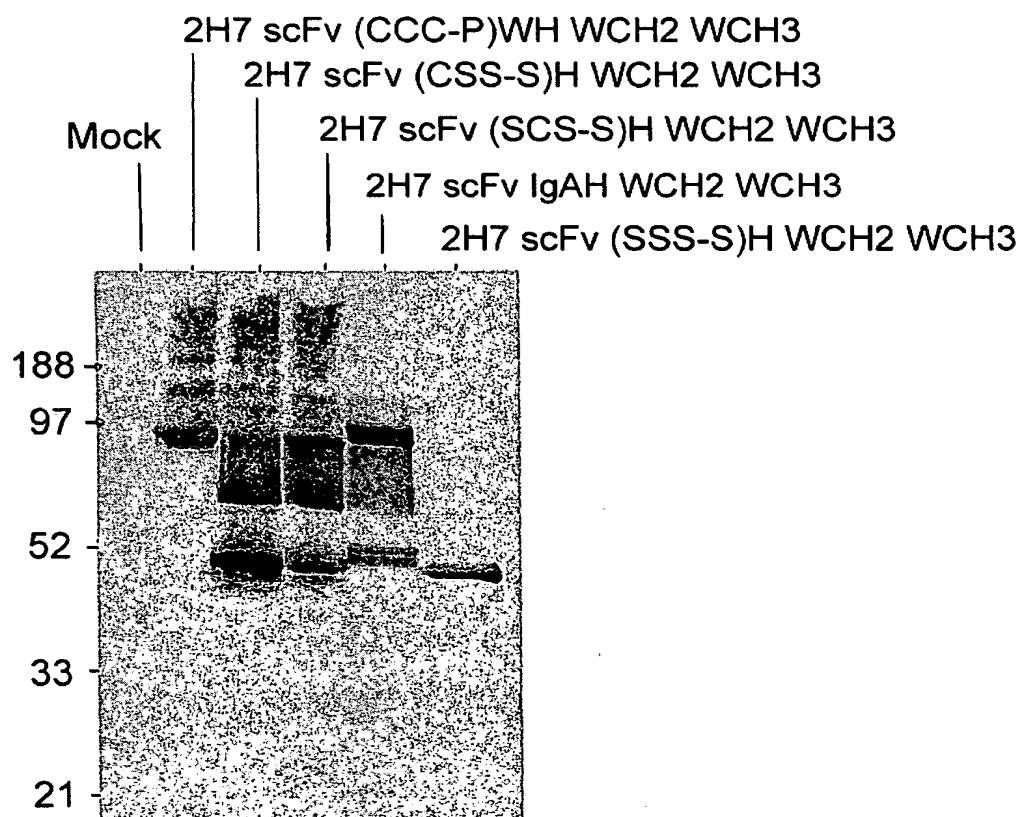
FIG.35

FIG.36

Binding to CD20 CHO cells by Constructs
that link anti-CD20 scFv to IgA Fc Domains

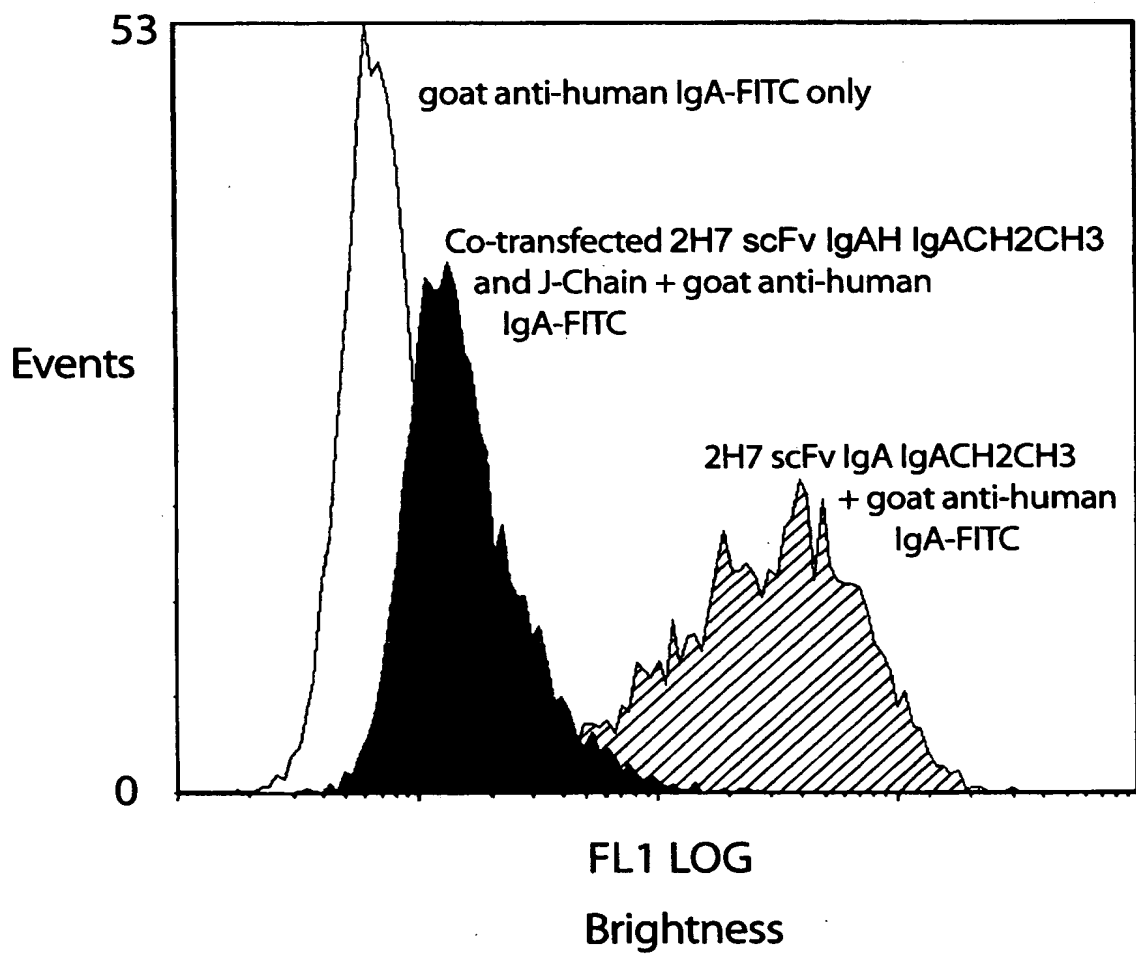


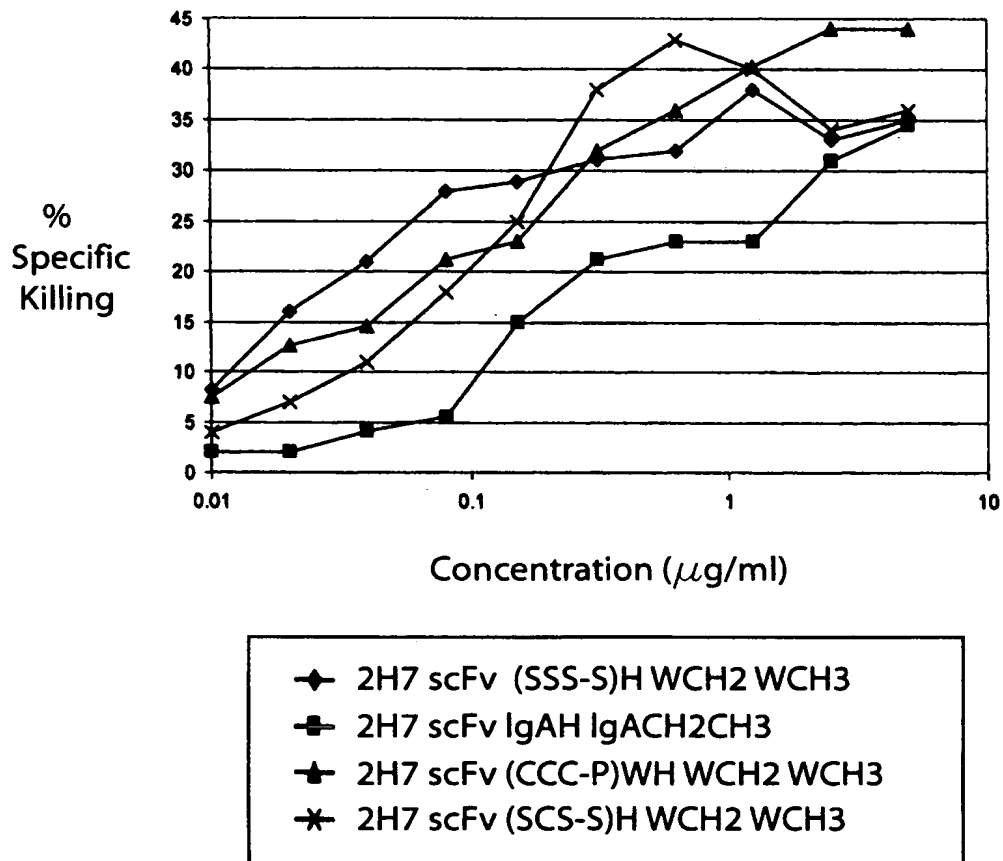
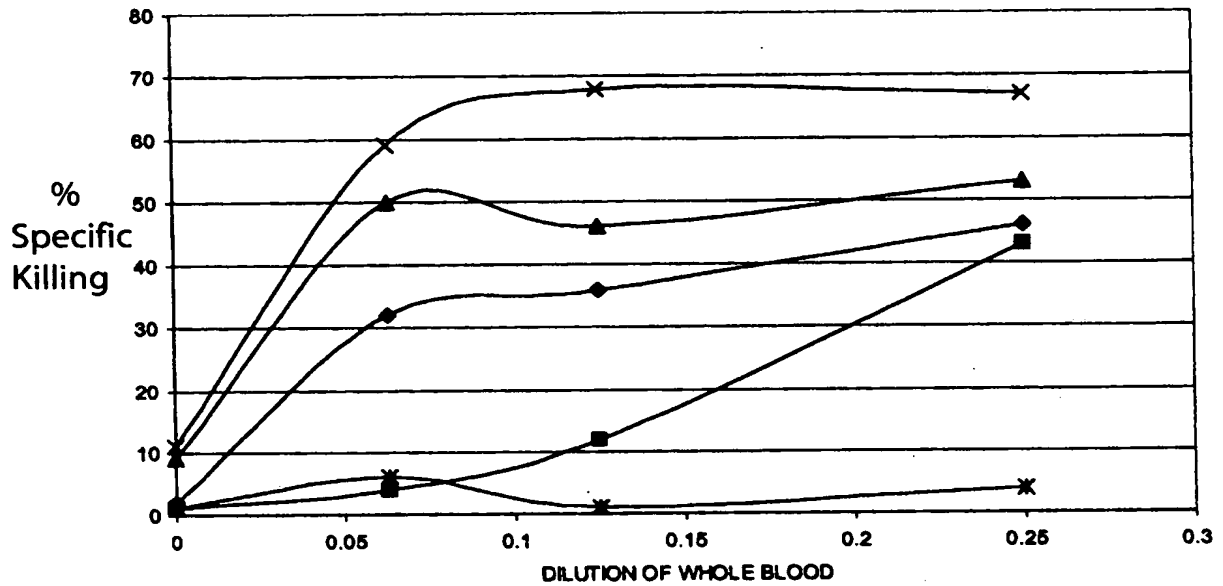
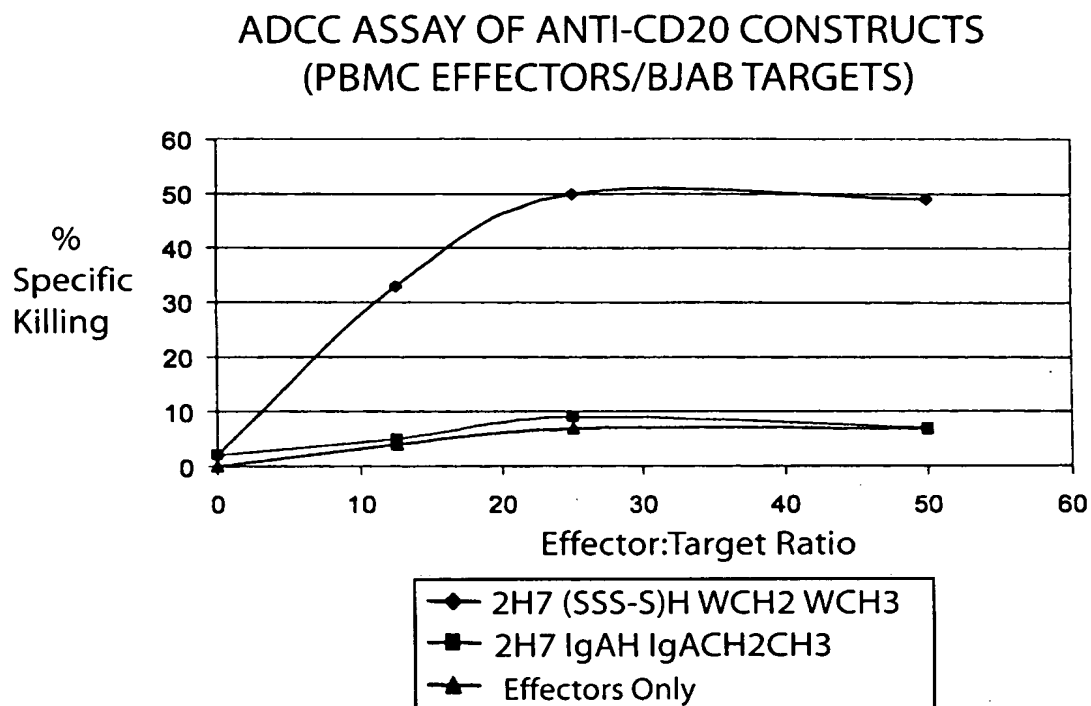
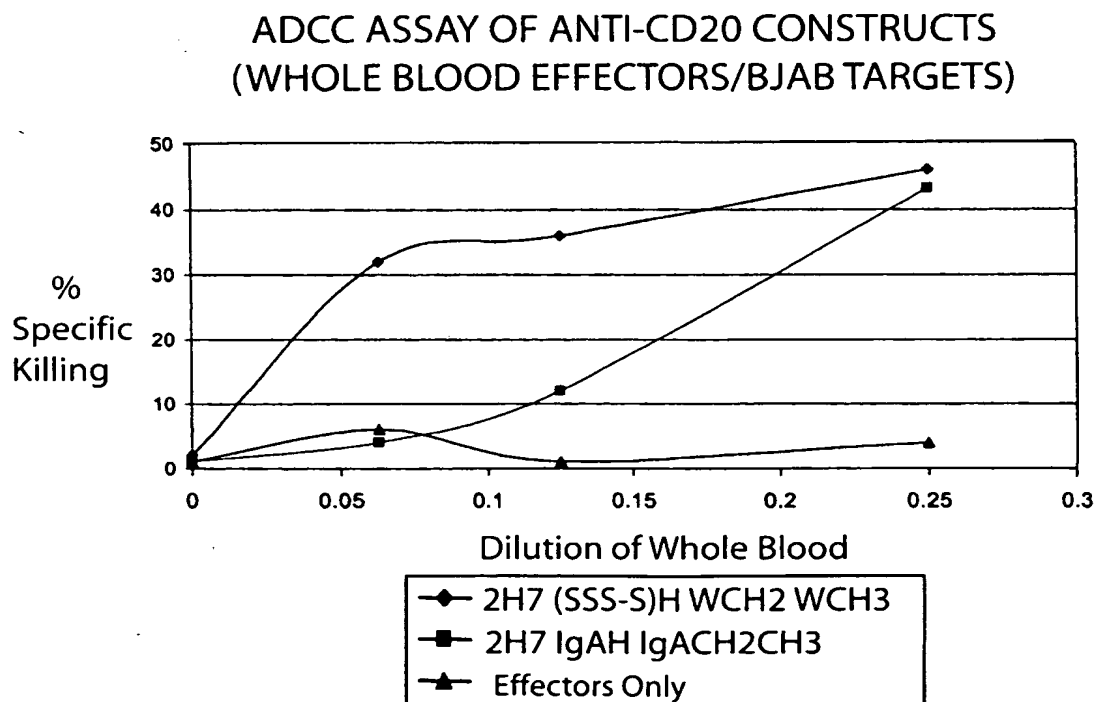
FIG.37**Titration of CD20 Specific scFvlg Constructs for ADCC Activity Using Whole Blood Effectors**

FIG.38

ADCC ASSAY OF ANTI-CD20 CONSTRUCTS WITH DIFFERENT TAILS
(WHOLE BLOOD EFFECTORS/BJAB TARGETS)



- ◆ 2H7 scFv (SSS-S)H WCH2 WCH3
- 2H7 IgAH IgACH2CH3
- ▲ 2H7 scFv (SCS-S)H WCH2 WCH3
- ✕ 2H7 scFv (CCC-P)WH WCH2 WCH3
- ✱ Effectors Only

FIG.39A**FIG.39B**

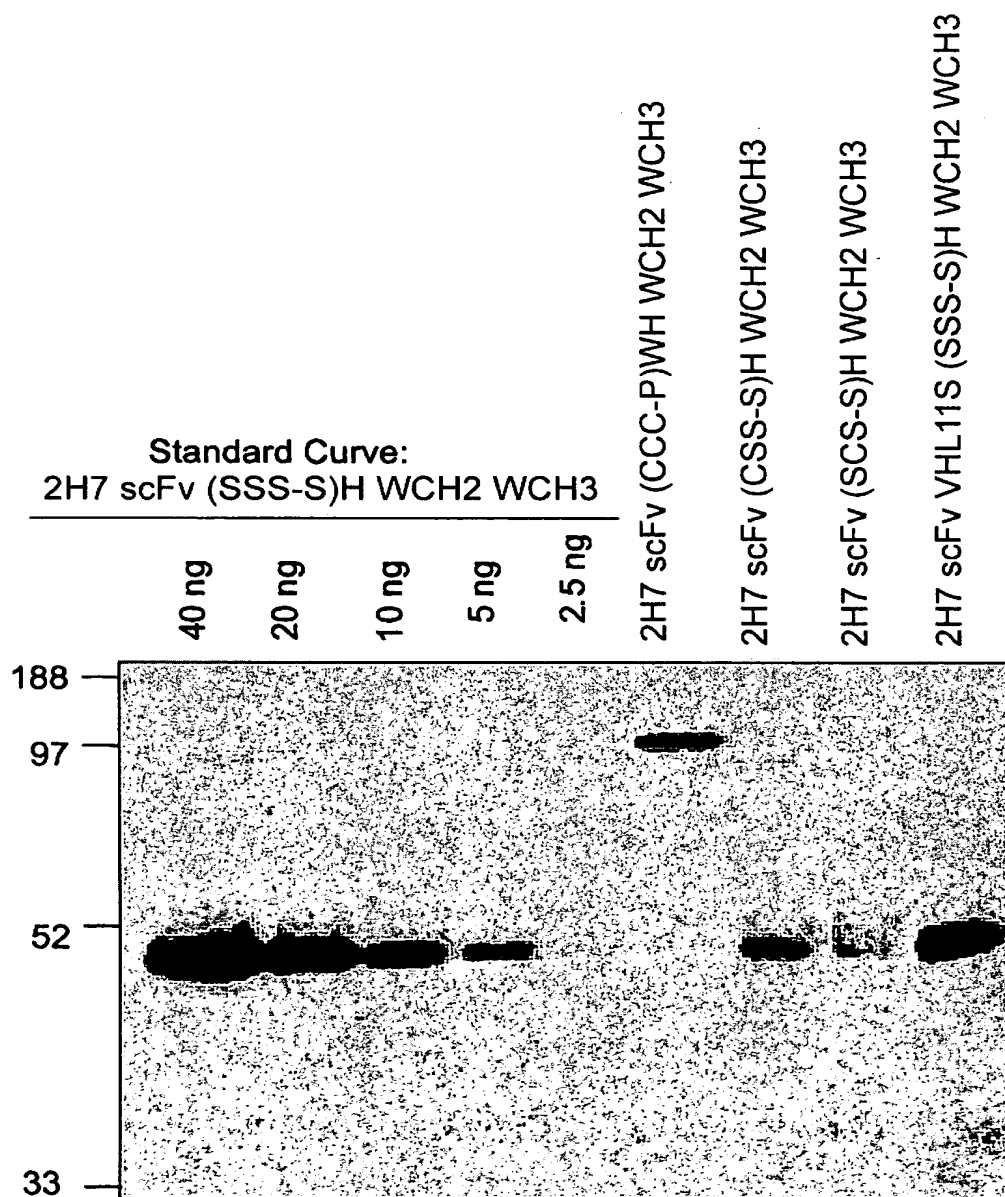


FIG.41A

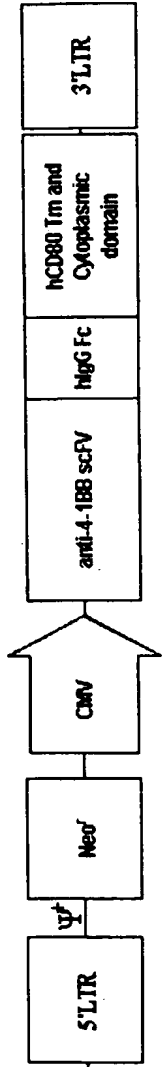


FIG.41B

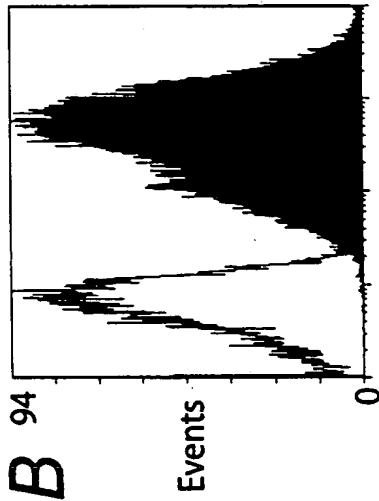


FIG.41C

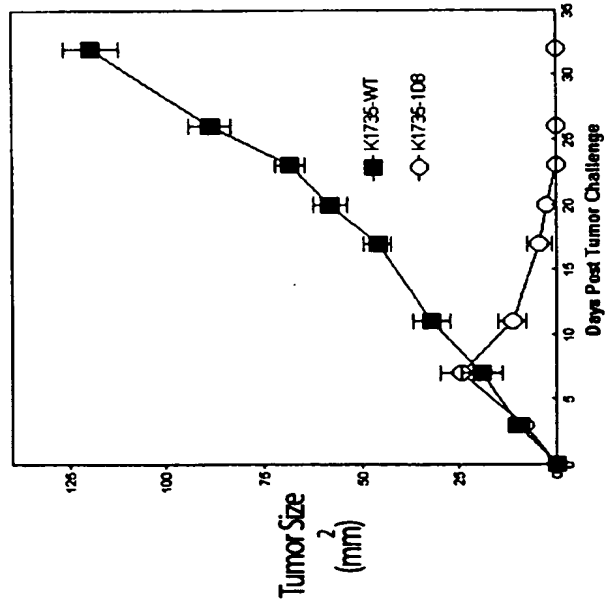


FIG.41D

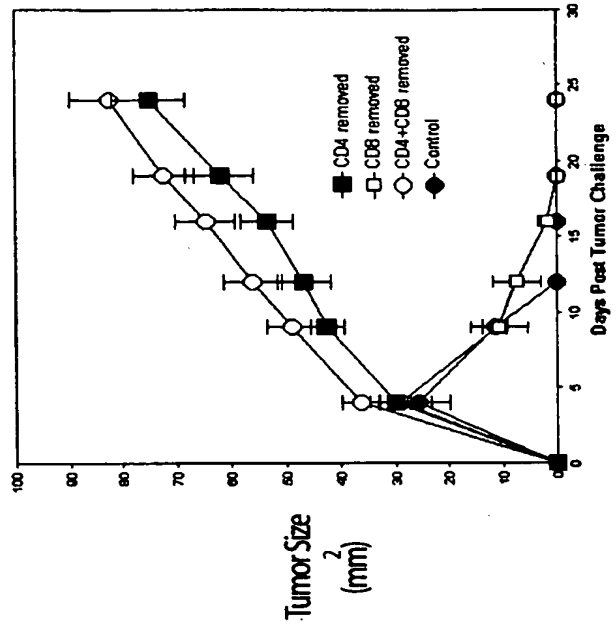


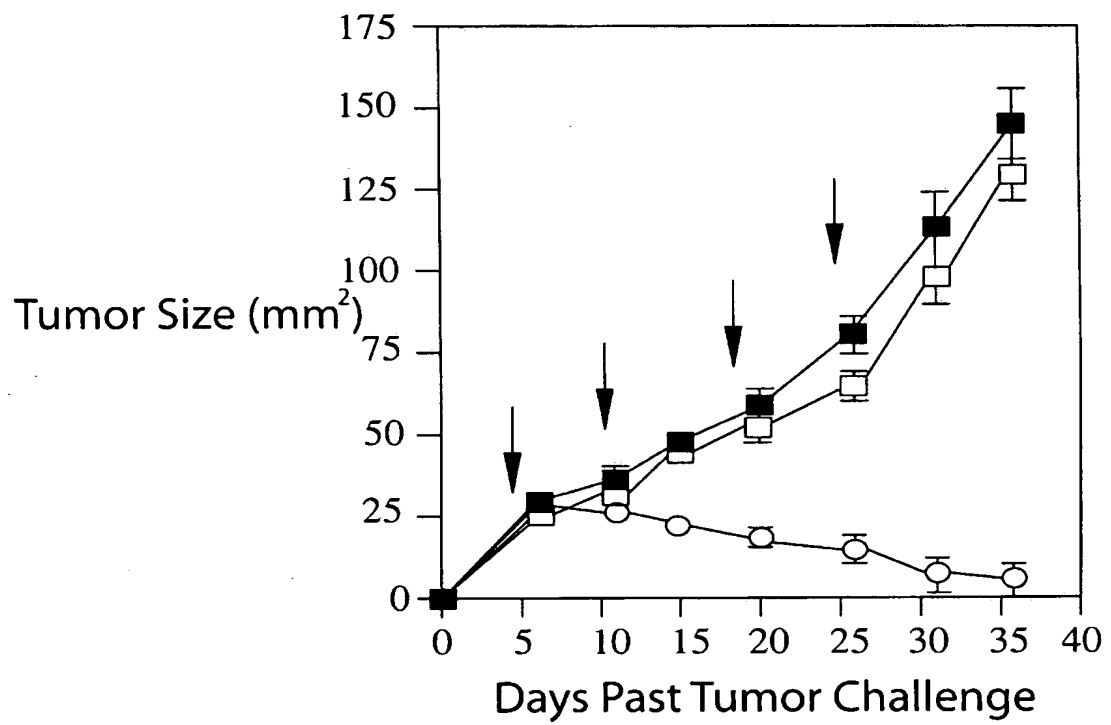
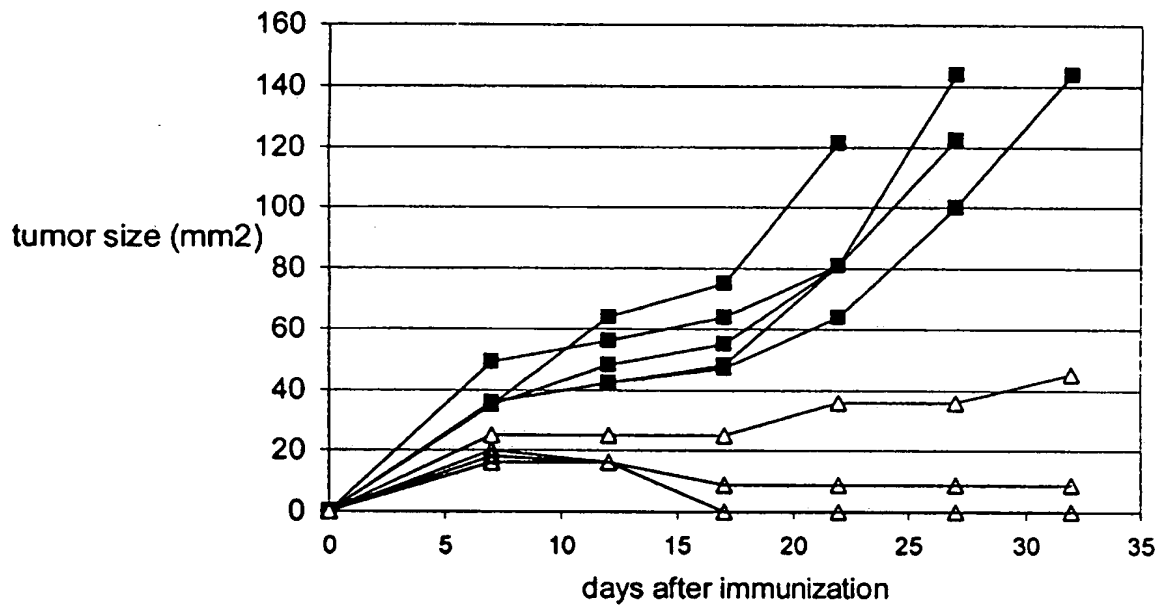
FIG.42

FIG.43

Mixtures of K1735-WT and K1735-1D8 transfected tumor lines
inhibit tumor outgrowth in C3H mice



- K1735-WT 1446
- K1735-WT 1422
- K1735-WT 1447
- K1735-WT 1420
- K1735-WT 1421
- △ K1735-WT+10%K1735-1D8 1492
- △ K1735-WT+10%K1735-1D8 1699
- △ K1735-WT+10%K1735-1D8 1493
- △ K1735-WT+10%K1735-1D8 1496

FIG.44

Expression of 1D8 scFv (SSS-S)H P238SCH2 WCH3 (Anti-CD37)
on the Surface of Panned Ag104 Transfected Tumor Cells

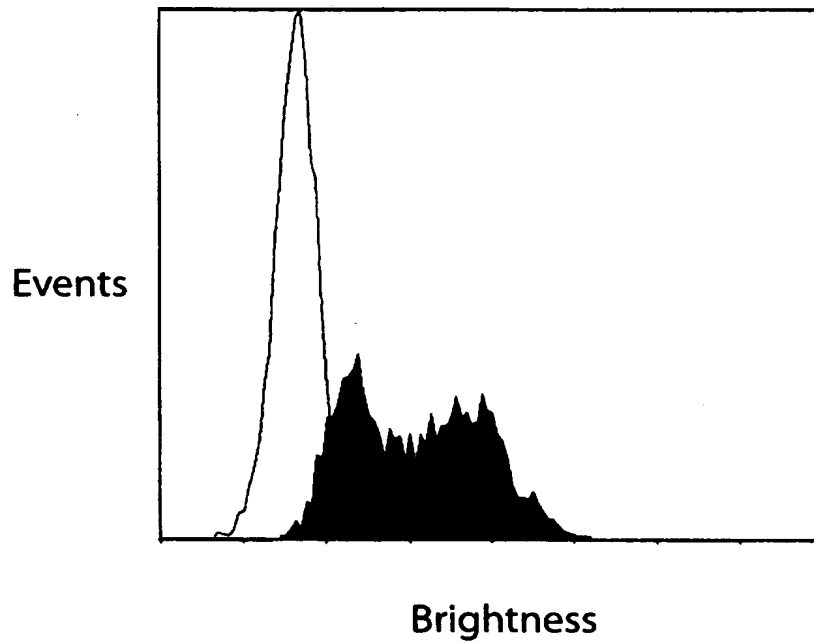


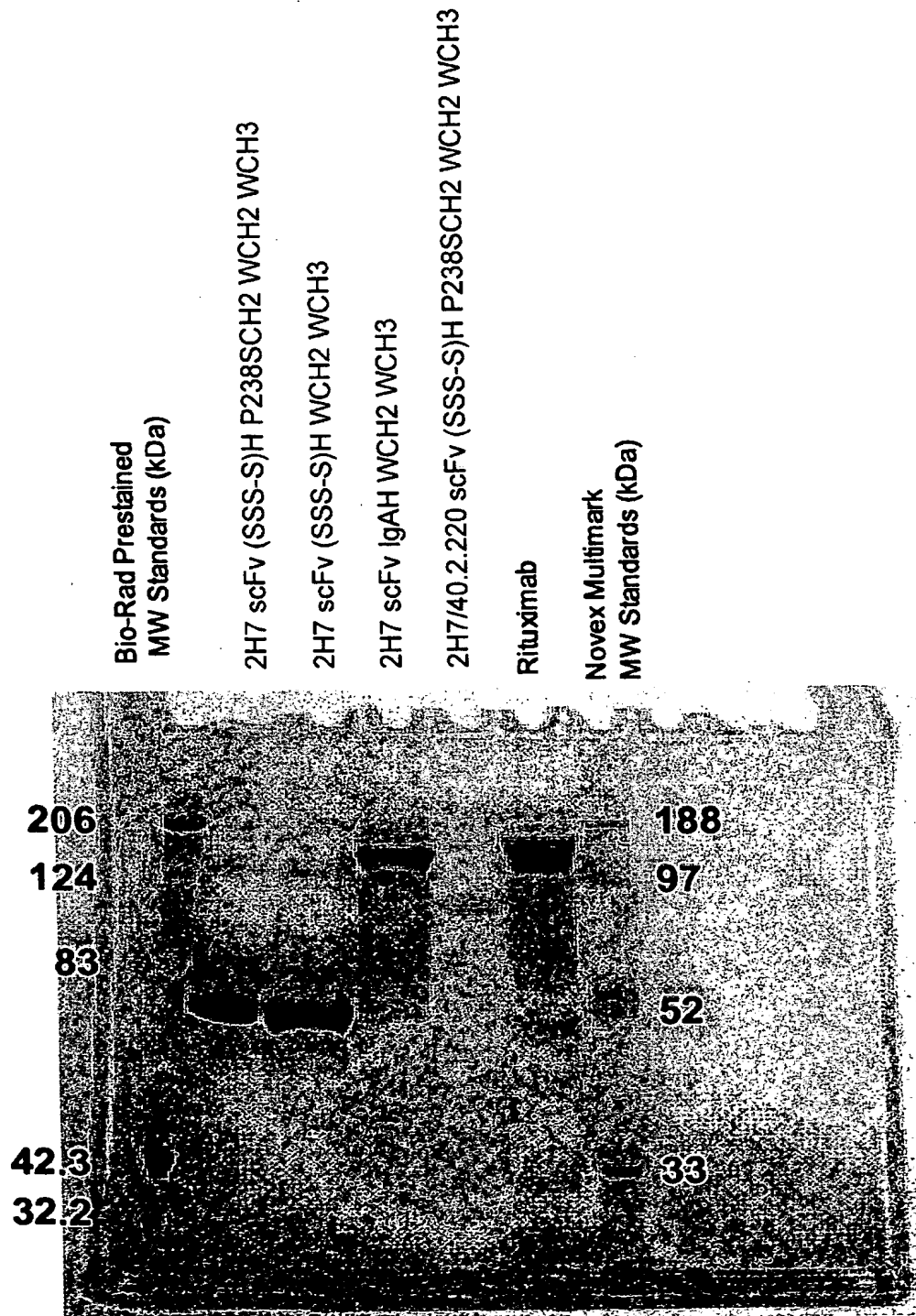
FIG.45

FIG.46

ADCC mediated by 2H7 scFvIg constructs
by human PBMC effector cells against Bjab targets

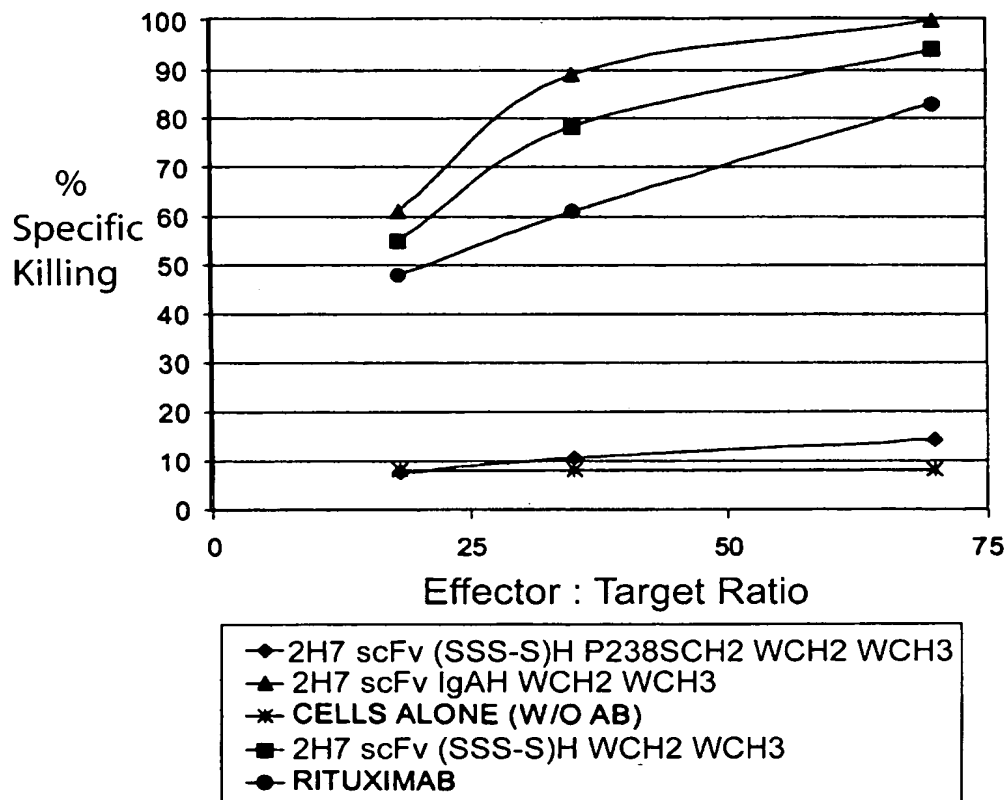


FIG.47

Cell surface expression of
G19-4scFv (SSS-S)H P238SCH2 WCH3
fusion protein on Reh and T51 Cells.

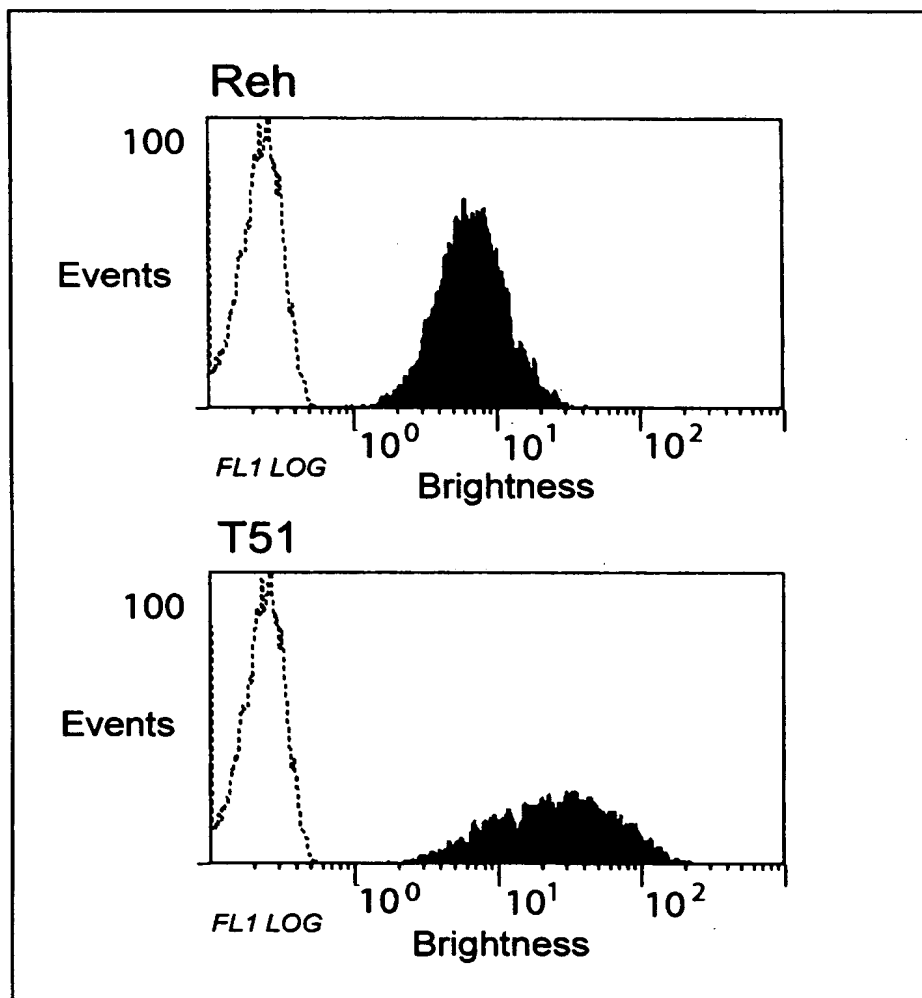


FIG.48

Targeting of Cytotoxicity to Transfected Cell Lines
by Surface expression of
G19-4 scFv (SSS-S)H P238SCH2 WCH3

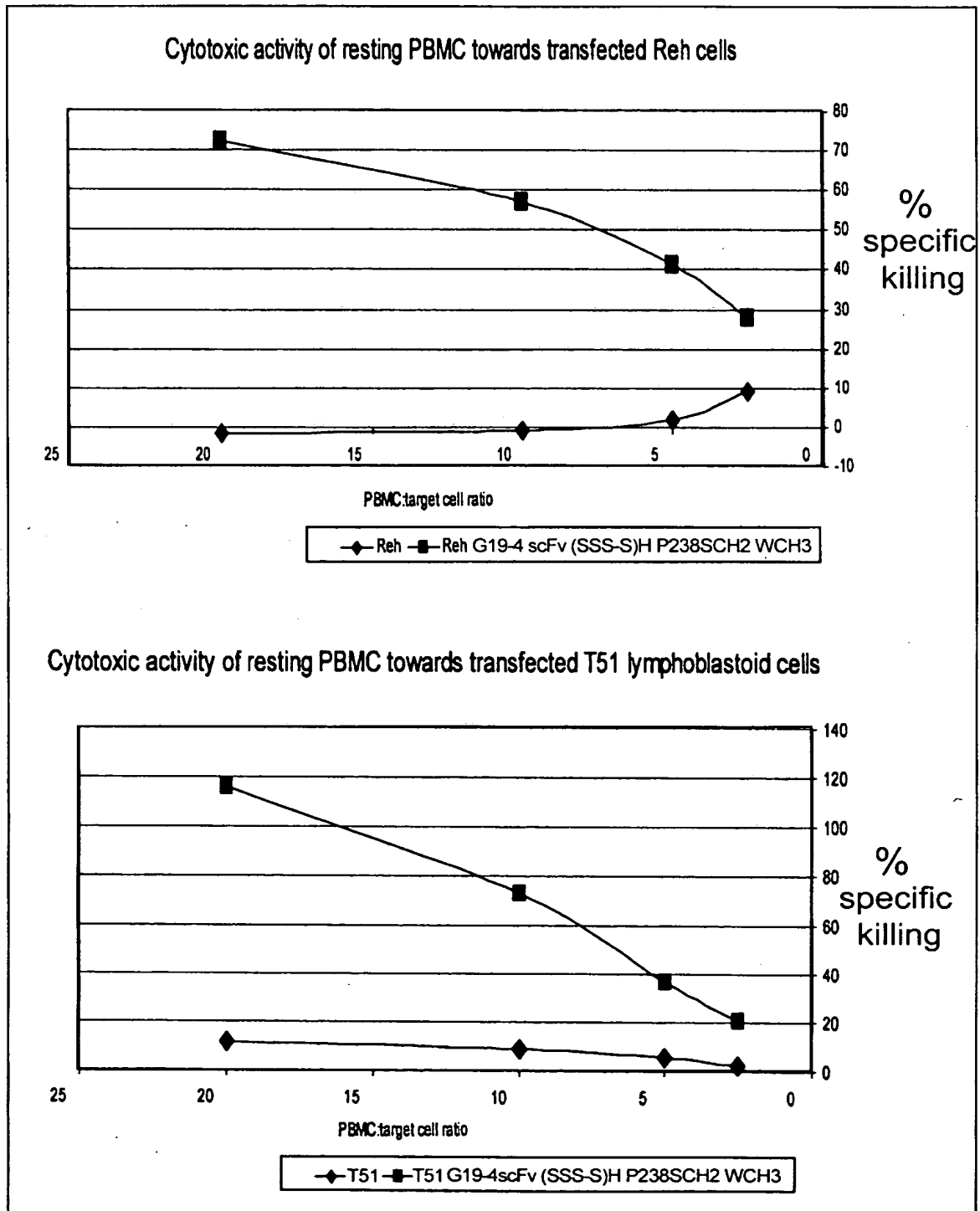


FIG.49

Binding of 5B9 scFv (SSS-S)H WCH2 WCH3,
a mouse anti-human CD137 to stimulated human PBMC

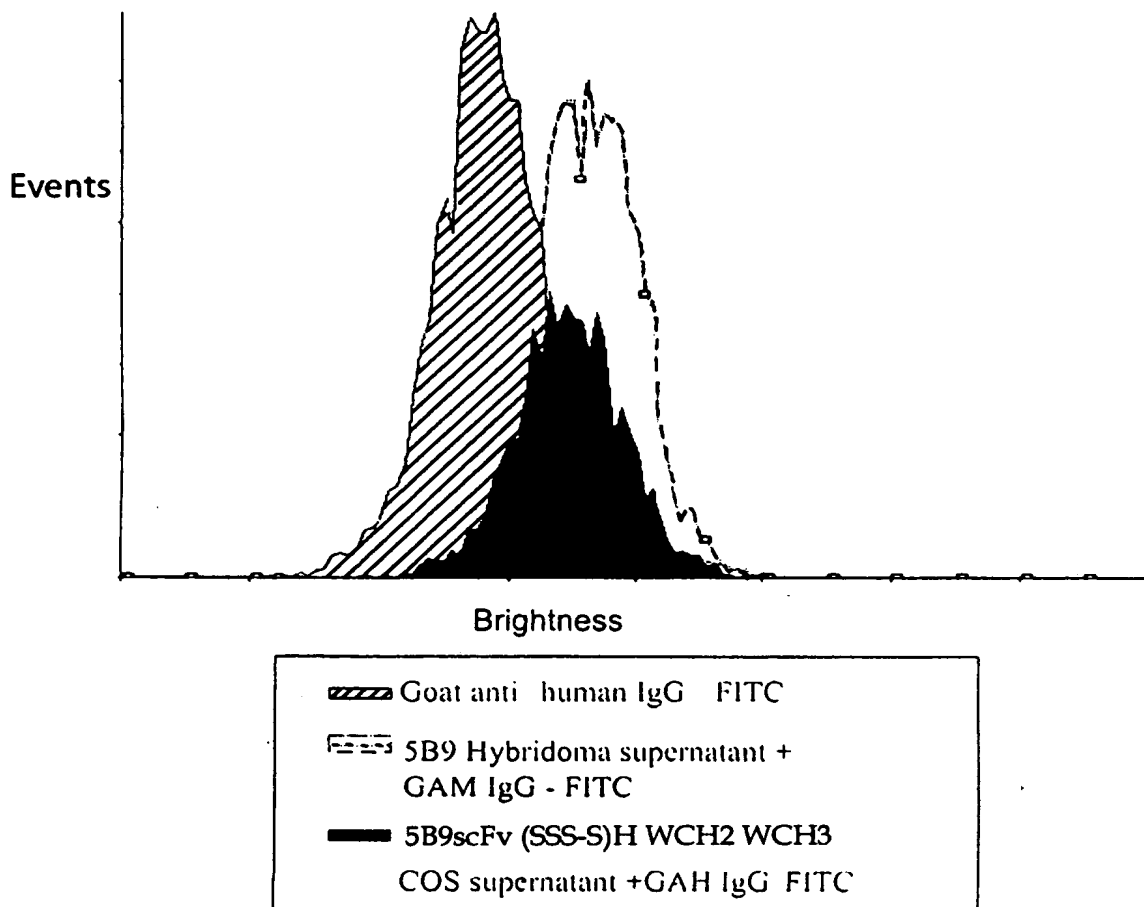
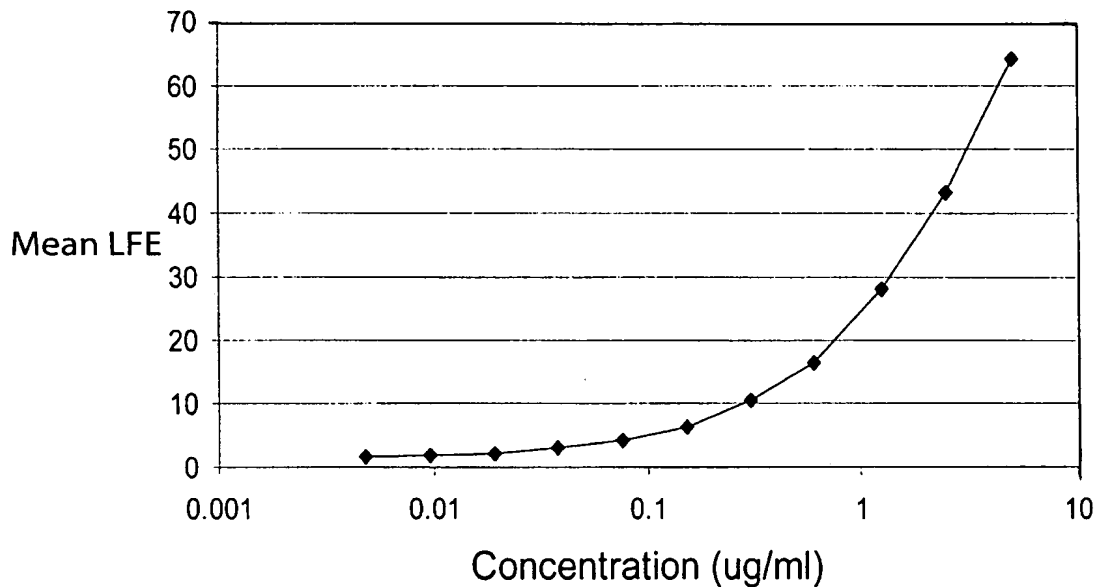


FIG.50A

Effect of V_HL11S Mutation on
2H7 scFv (SSS-S)H WCH2 WCH3 Protein Expression

Standard Curve: 2H7scFv VHL11S (SSS-S)H WCH2 WCH3

**FIG.50B**

Effect of V_HL11S Mutation on
2H7 scFv (SSS-S)H WCH2 WCH3 Protein Expression

CHO supernatant Brightness and Estimation of Protein concentrations from Standard Curve:					
CHO clone name					
	<u>4F2</u>	<u>4F5</u>	<u>3E5</u>	<u>6B11A</u>	<u>2B8A</u>
Mean LFE					
1/100	71.7	40.6	31.5	99.7	101.5
1/500	27.1	12.4	11.2	40.8	43
approx conc. µg/ml	600	225	125	1000	1250

FIG.51

**Production Levels of 2H7scFv VH L11S
(SSS-S)H WCH2 WCH3
From CHO Clone Culture Supernatants**

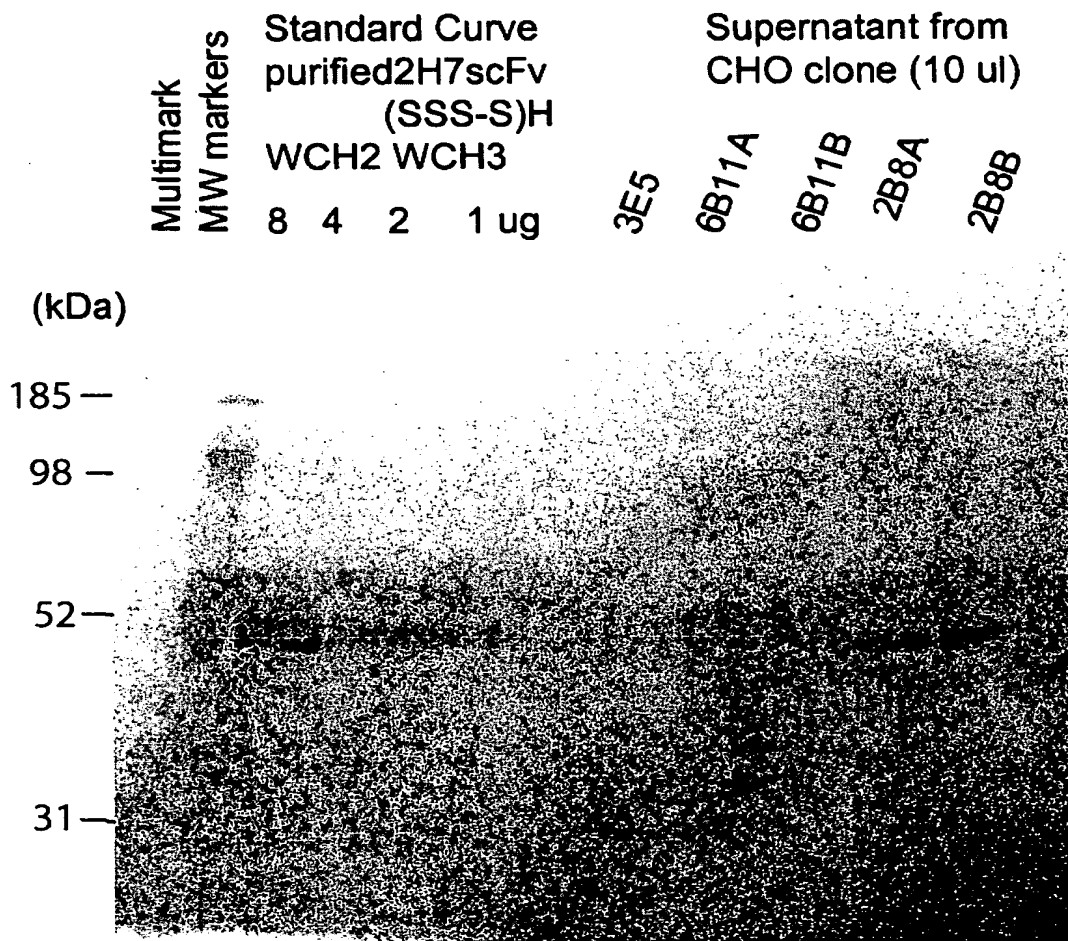


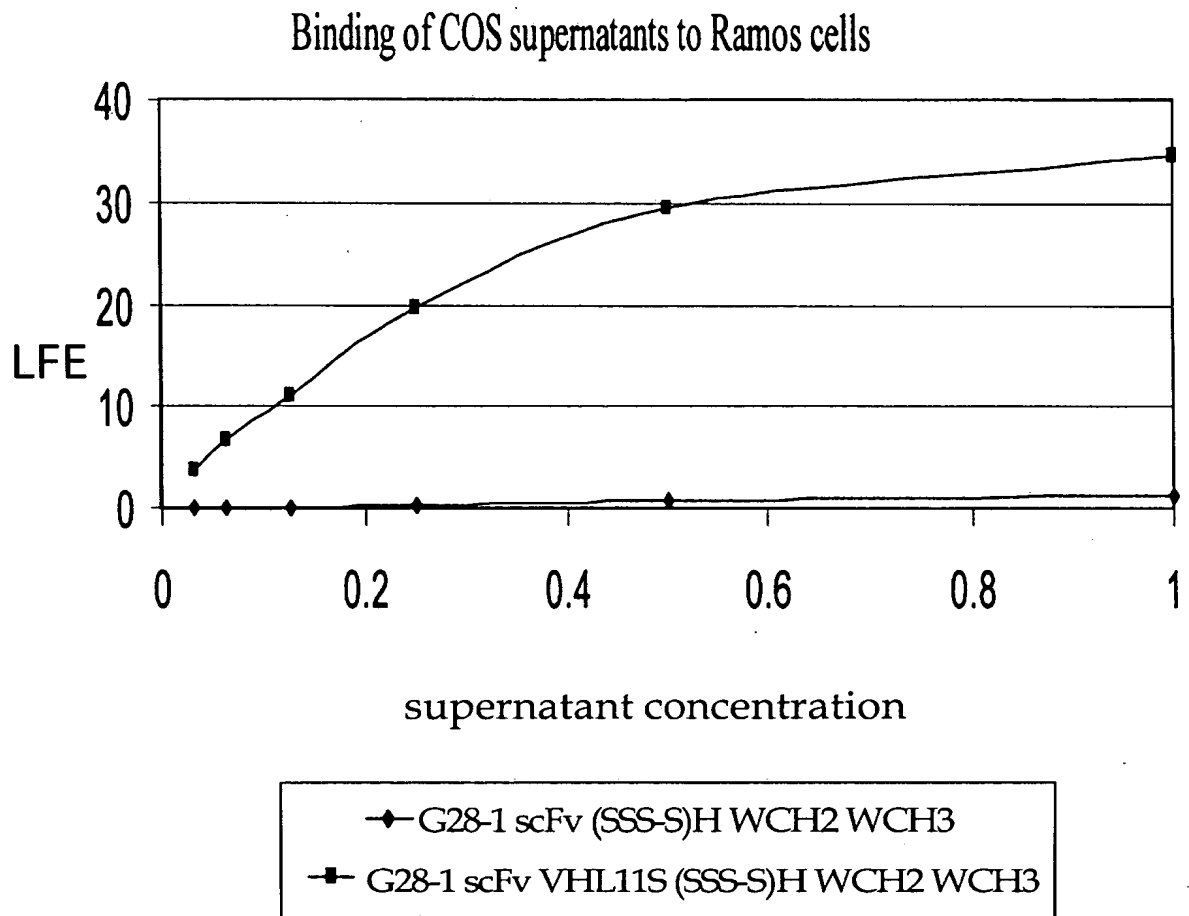
FIG.52**Effect of VHL11S Mutation on G28-1 scFvIg Construct
Protein Production from COS cells**

FIG. 53A

Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants
transfected with G28-1scFv (SSS-S)H WCH2 WCH3
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

Purified G28-1 G28-1 scFv
scFv (SSS-S)H (SSS-S)H
WCH2 WCH3 WCH2 WCH3
1 ul/well

80ng 40ng 20ng 10ng A B C D E

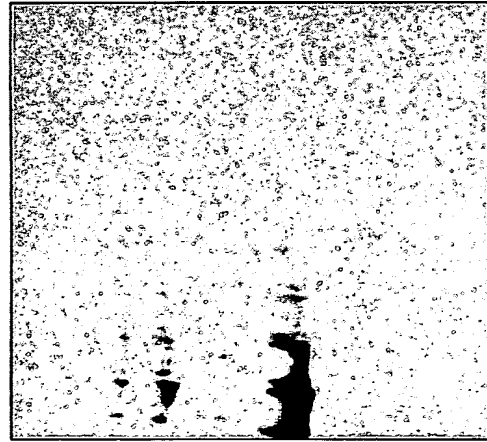


FIG. 53B

Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants
transfected with G28-1scFv (SSS-S)H WCH2 WCH3
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

Purified G28-1 G28-1 scFv VHL11S
scFv (SSS-S)H WCH2 WCH3
WCH2 WCH3 1 ul/well

80ng 40ng 20ng 10ng A B C D E

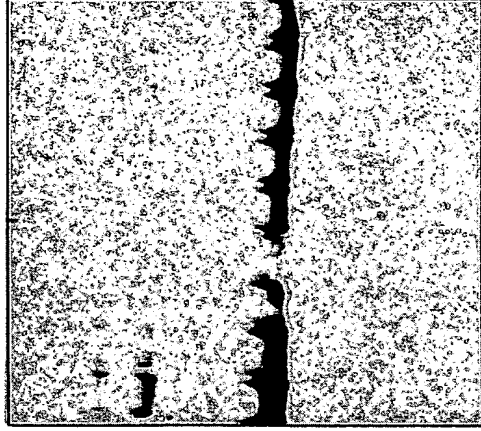
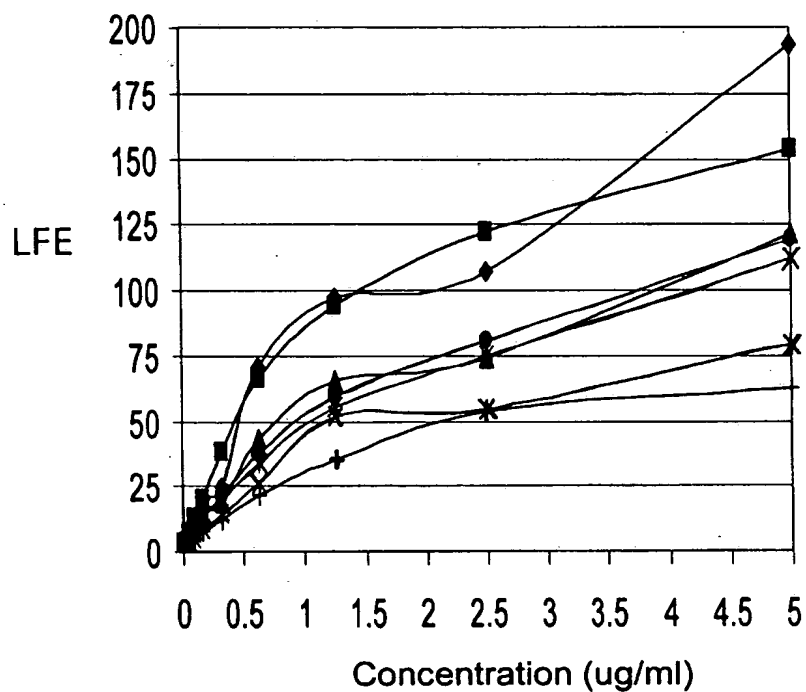


FIG.54

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Binding of 2H7 scFvIg Constructs with Altered Hinges and CH3 domains to CD20 CHO Cells

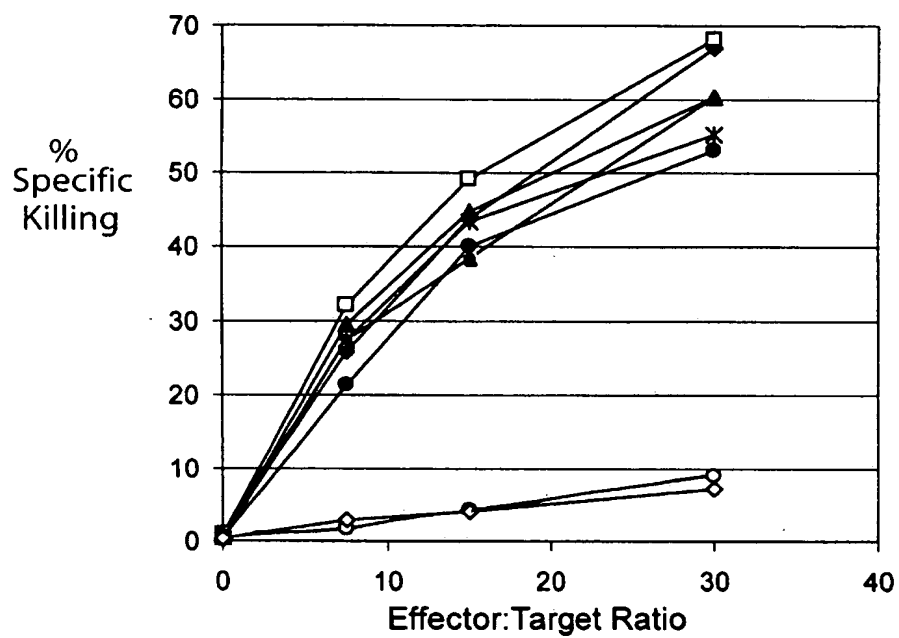


- ◆ 2H7 scFv VHL11S
(SSS-S)H WCH2 WCH3
- 2H7 scFv (SSS-S)H
WCH2 Y407ACH3
- ▲ 2H7 scFv (SSS-S)H
WCH2 WCH3
- ✕ 2H7 scFv (CSS-S)H
WCH2 WCH3
- ✱ 2H7 scFv (CCC-P)WH
WCH2 WCH3
- 2H7 scFv (SCS-S)H
WCH2 WCH3
- + 2H7 scFv (CSC-S)H
WCH2 WCH3

FIG.55

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**ADCC Activity of 2H7 scFvlg constructs Against
BJAB Targets and PBMC Effectors**



- ◆— 2H7 scFv (CCC-P)WH WCH2 WCH3
- 2H7 scFv (CSS-S)H WCH2 WCH3
- ▲— 2H7 scFv (SCS-S)H WCH2 WCH3
- 2H7 scFv (CSC-S)H WCH2 WCH3
- *— 2H7 scFv (SSS-S)H WCH2 WCH3
- 2H7 scFv (SSS-S)H WCH2 Y407ACH3
- 2H7 scFv IgAH WCH2 WCH3
- ◇— Natural Killing

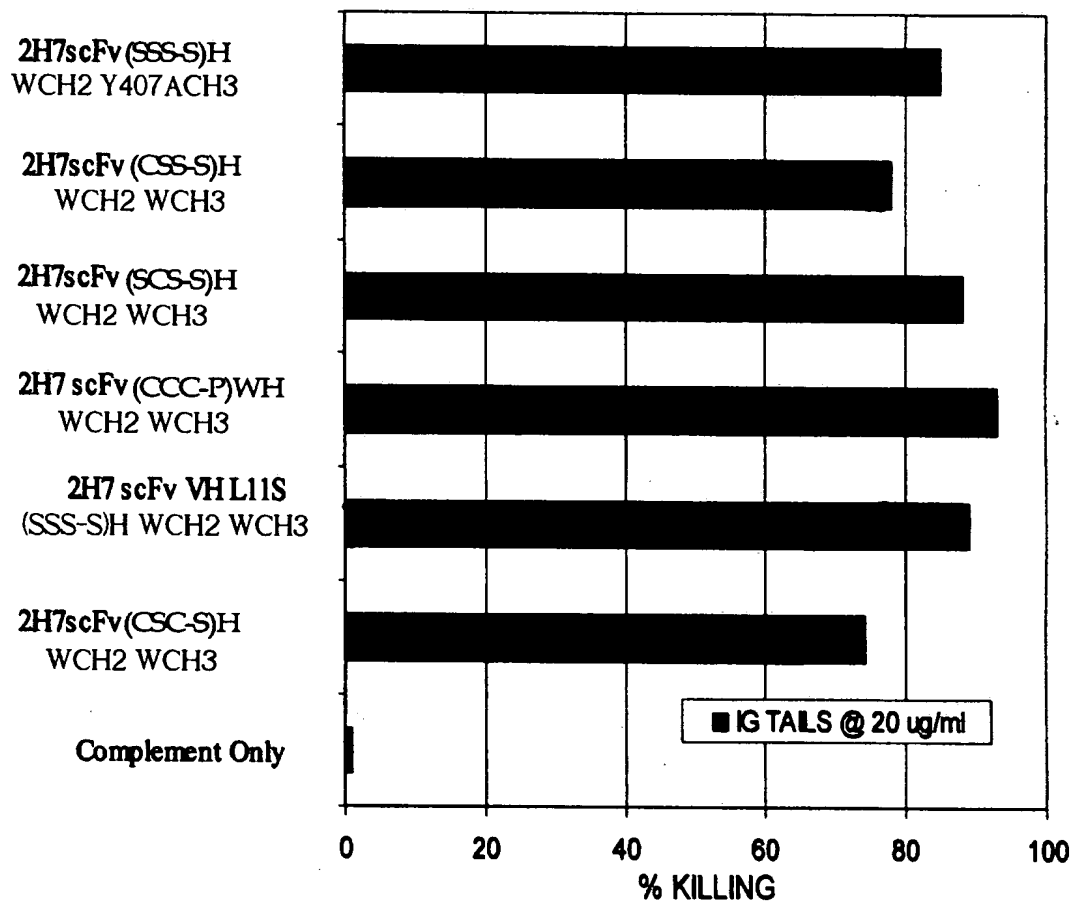
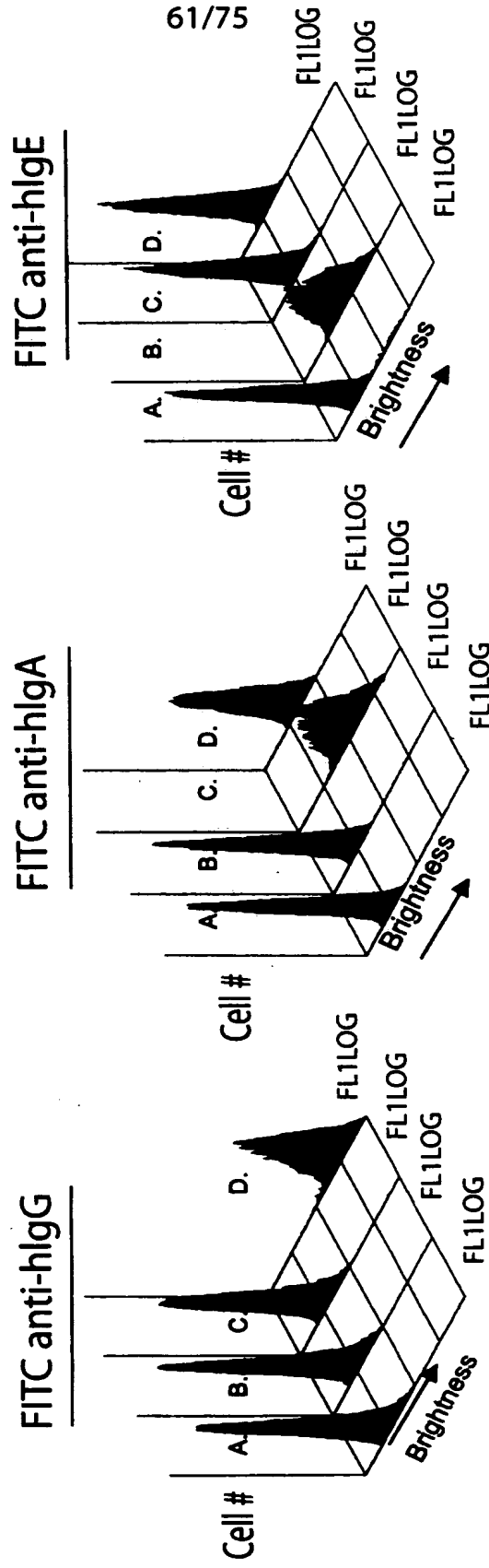
FIG.56**Complement Activity of 2H7 scFvlg Constructs
With Ramos Target Cells**

FIG.57

Binding of 2H7 scFvIg Derivatives CD20CHO Cells



- A. No fusion protein
- B. 2H7 scFv IgE CH2CH3CH4
- C. 2H7 scFv IgAH WCH2 WCH3
- D. 2H7 scFv(SSS-S)H WCH2 WCH3

FIG.58A

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2H7 scFv VH L11S human IgE CH2CH3CH4
Binding to CD20 CHO at 30 ug/ml

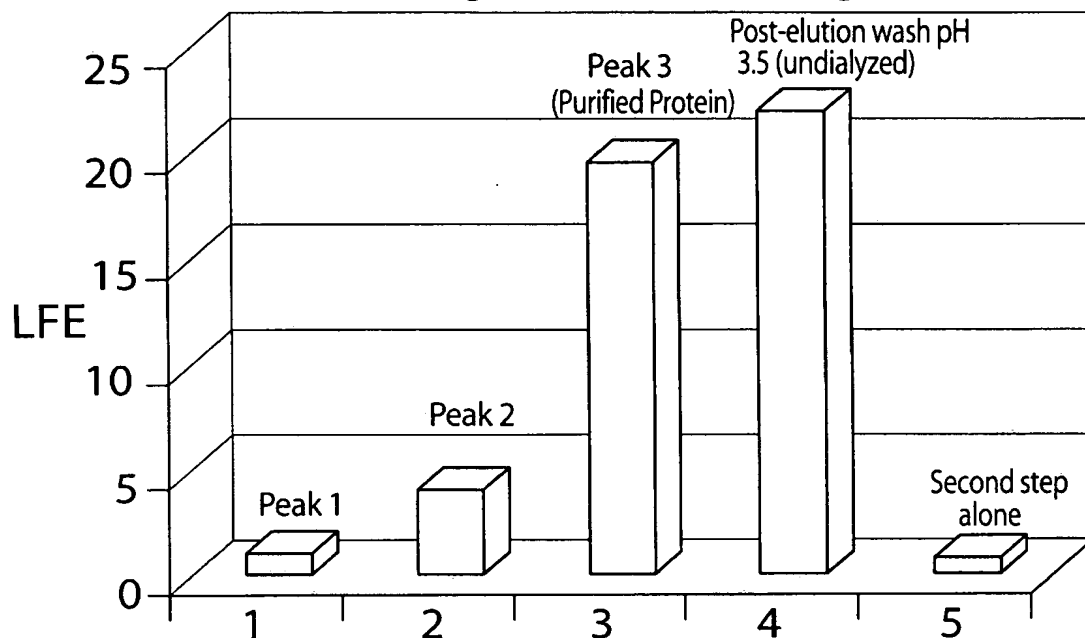


FIG.58B

ADCC Activity of 2H7 scFv VHL11S IgE CH2CH3CH4
Protein Fractions with PBMC Effectors and Bjab Targets

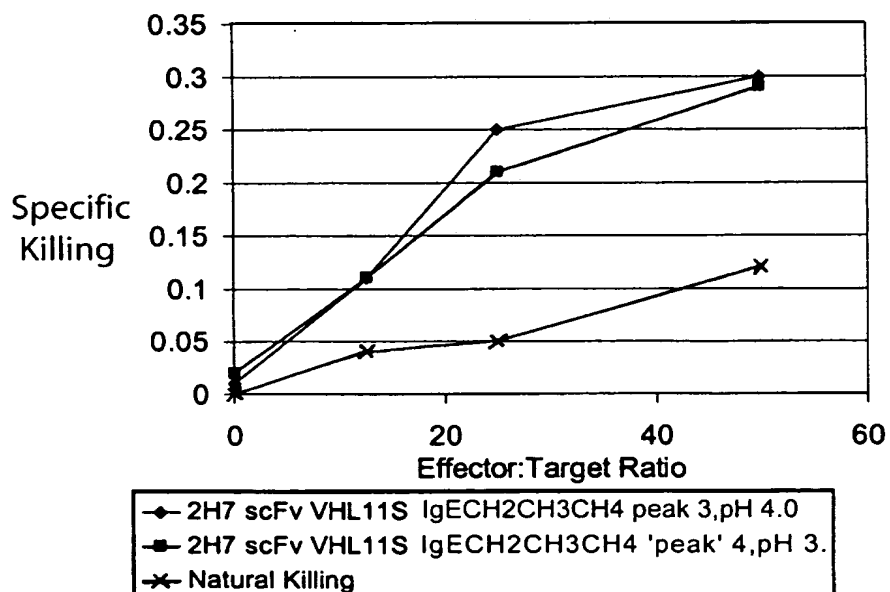


FIG.59

Binding Data For COS derived 2H7 scFv VHL11S
mIgECH2CH3CH4 and mIgAH WCH2 WCH3

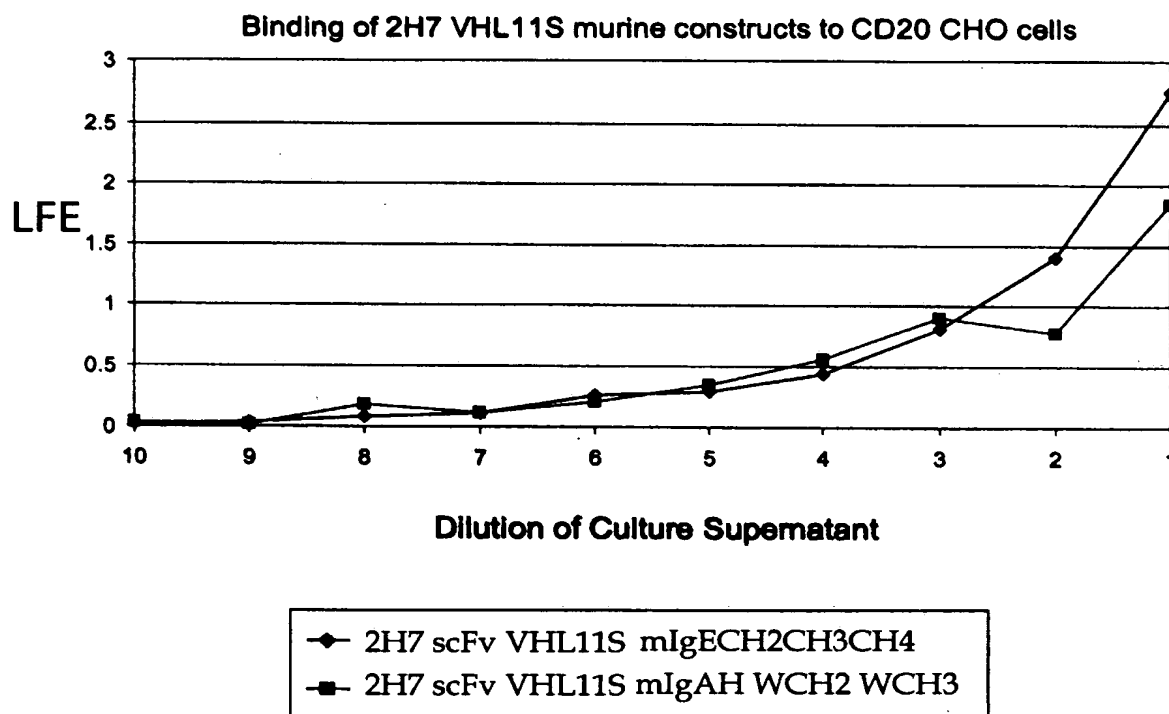


FIG. 60A

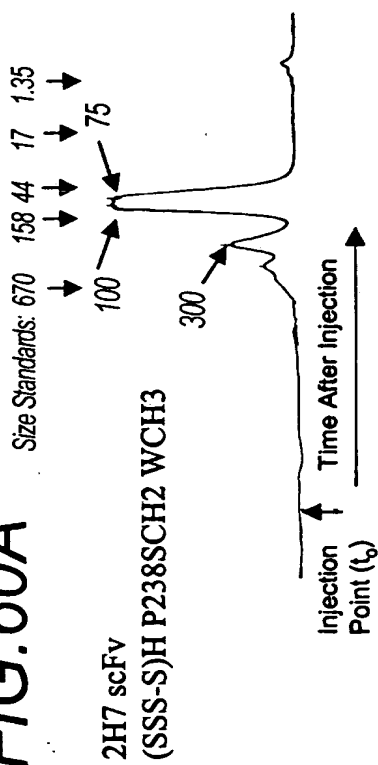


FIG. 60B

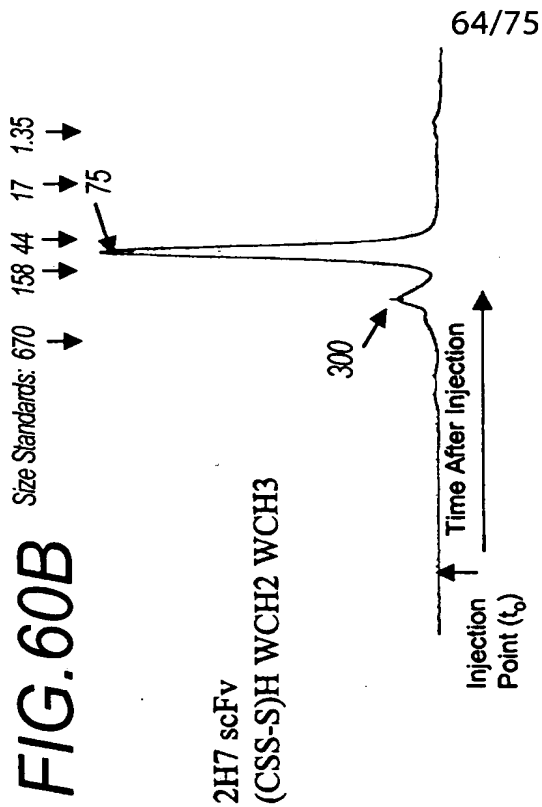


FIG. 60C

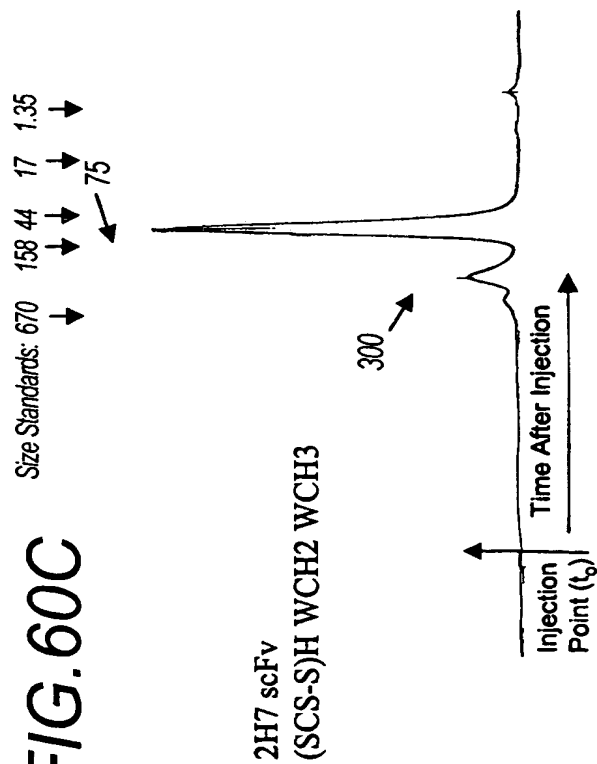


FIG. 60D

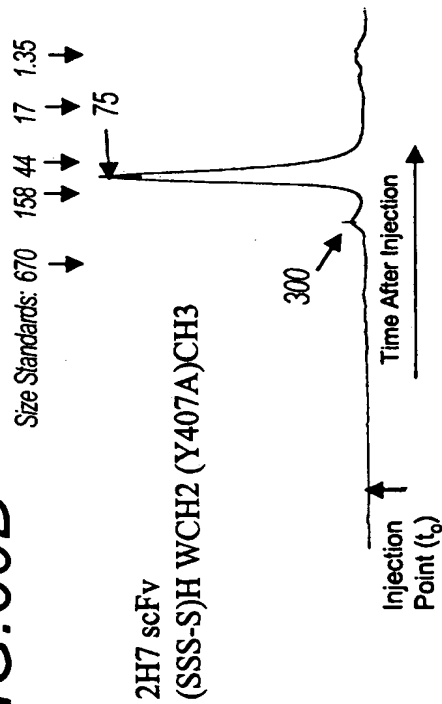


FIG. 61A

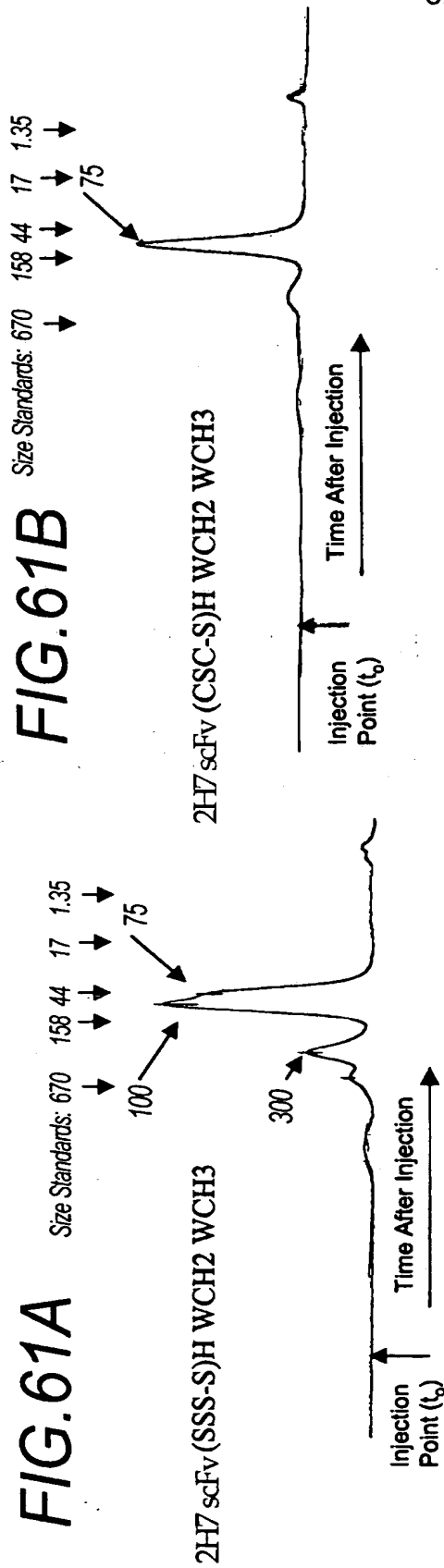


FIG. 61B

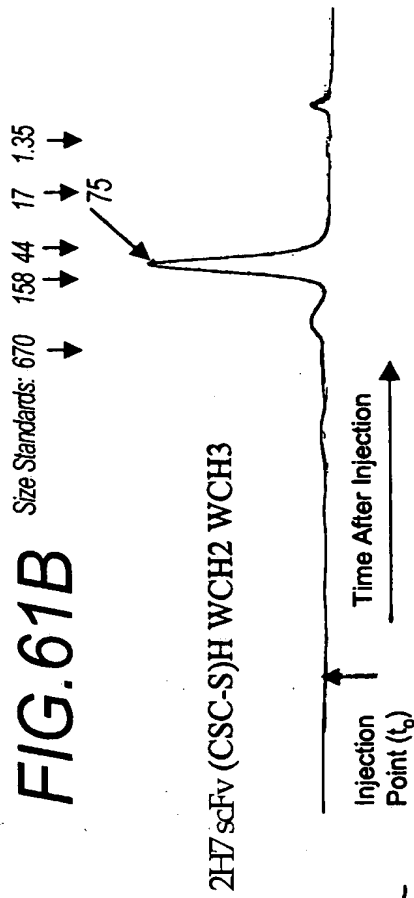


FIG. 61C

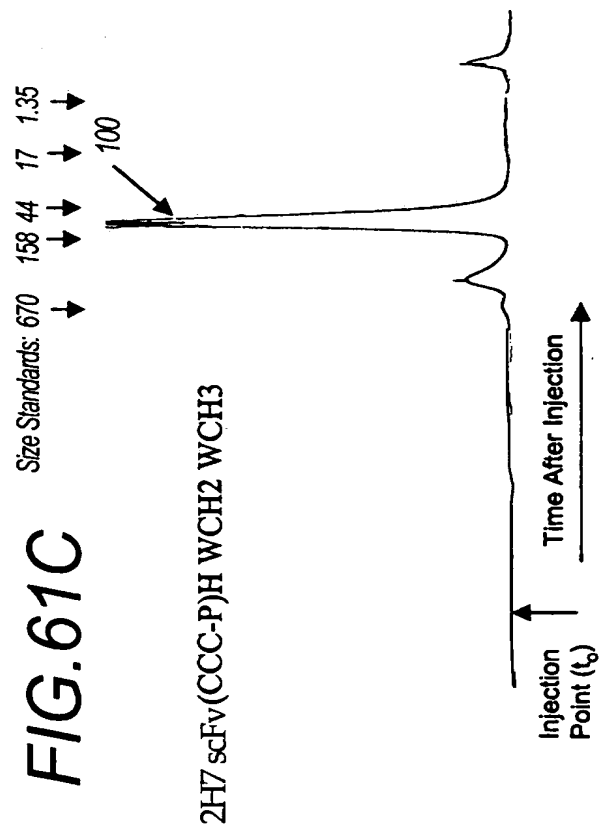
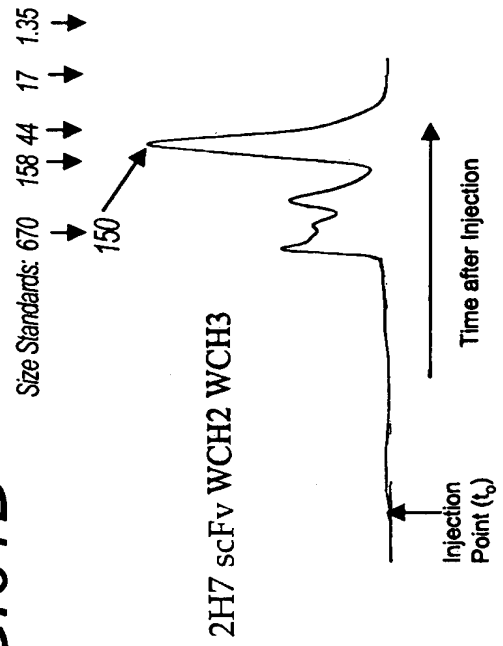


FIG. 61D



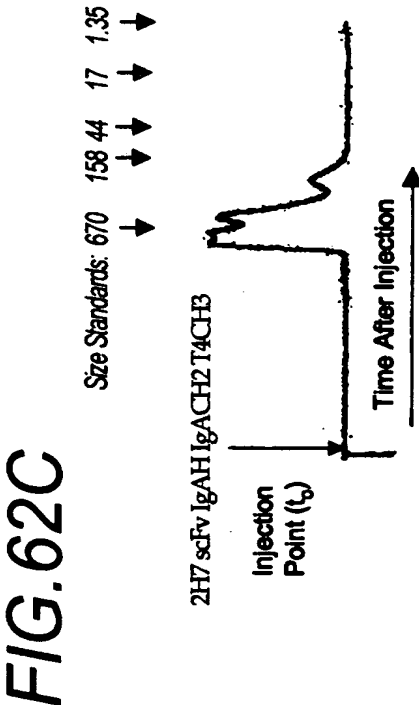
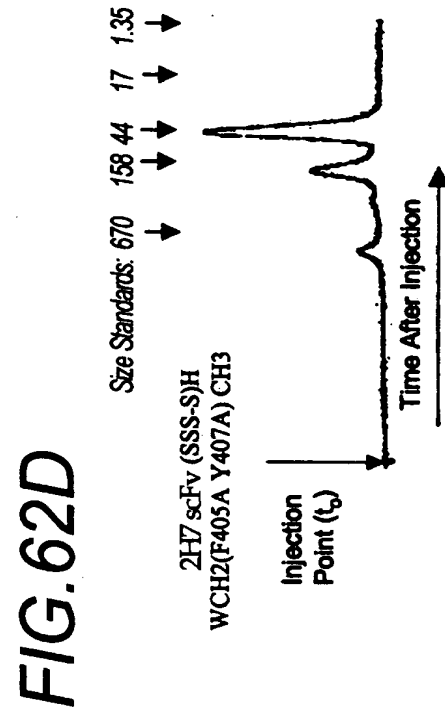
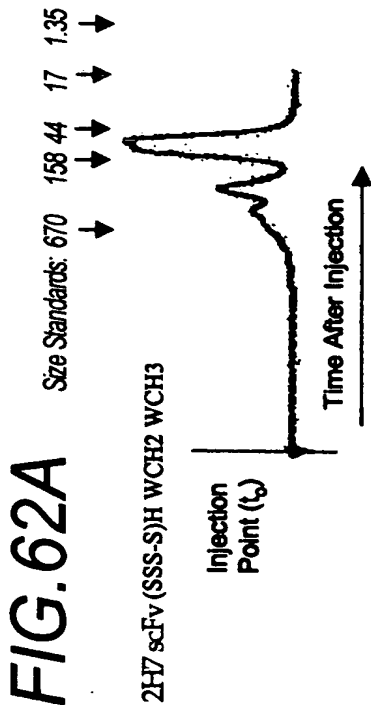
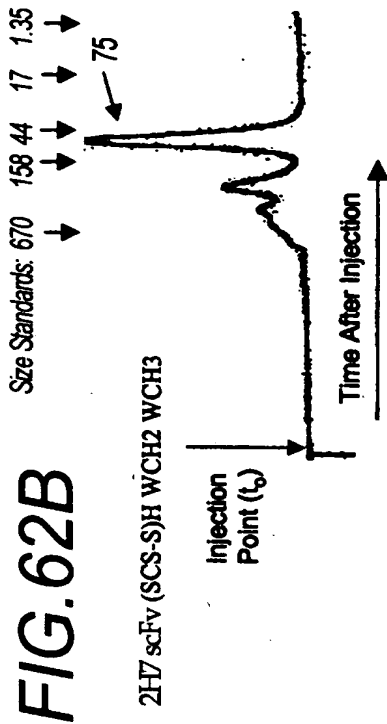


FIG.63

Binding of Purified Proteins from COS Supernatants
to CD20 CHO cells:
Differential Effects of CH3 Mutations on Binding

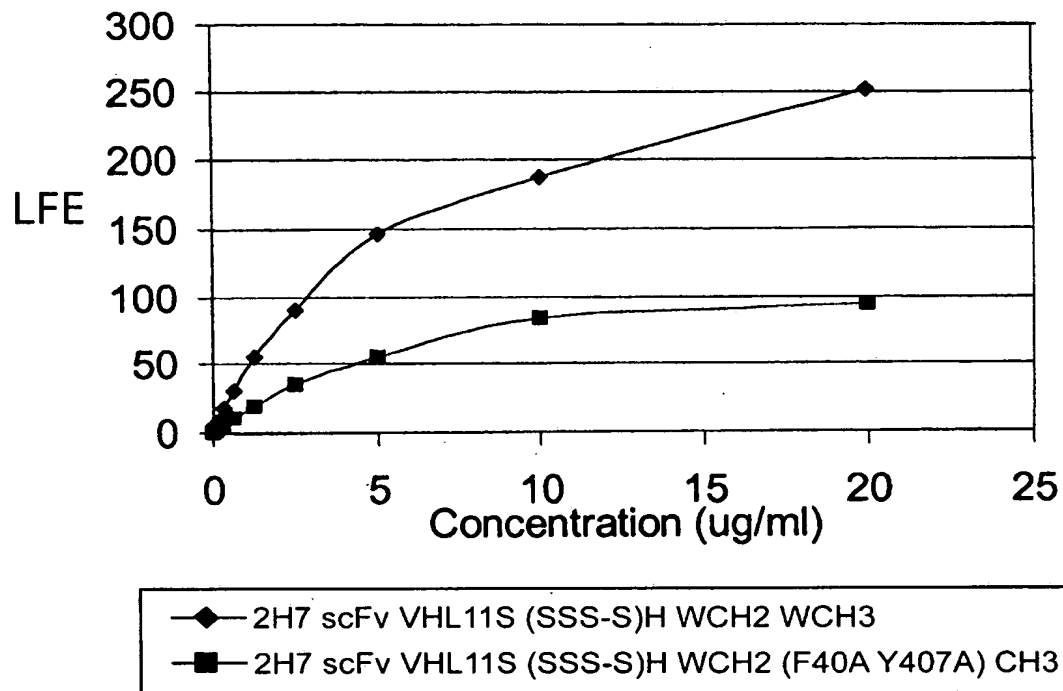


FIG.64

Binding of FITC conjugated 2H7 scFv VHL11S Proteins to
CD20 CH0 Cells

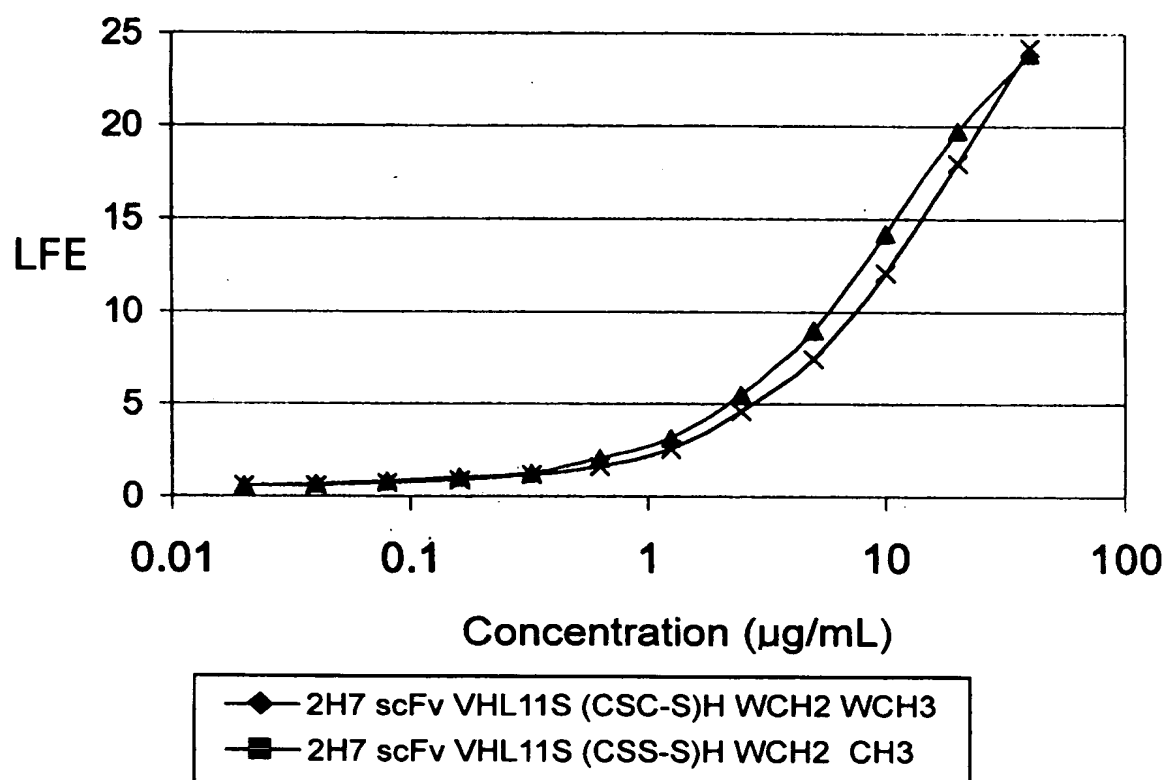


FIG. 65

Nonreducing SDS-PAGE on Protein A-Purified Lots
of 2H7 scFv VHL11S Constructs (10 ug/lane)

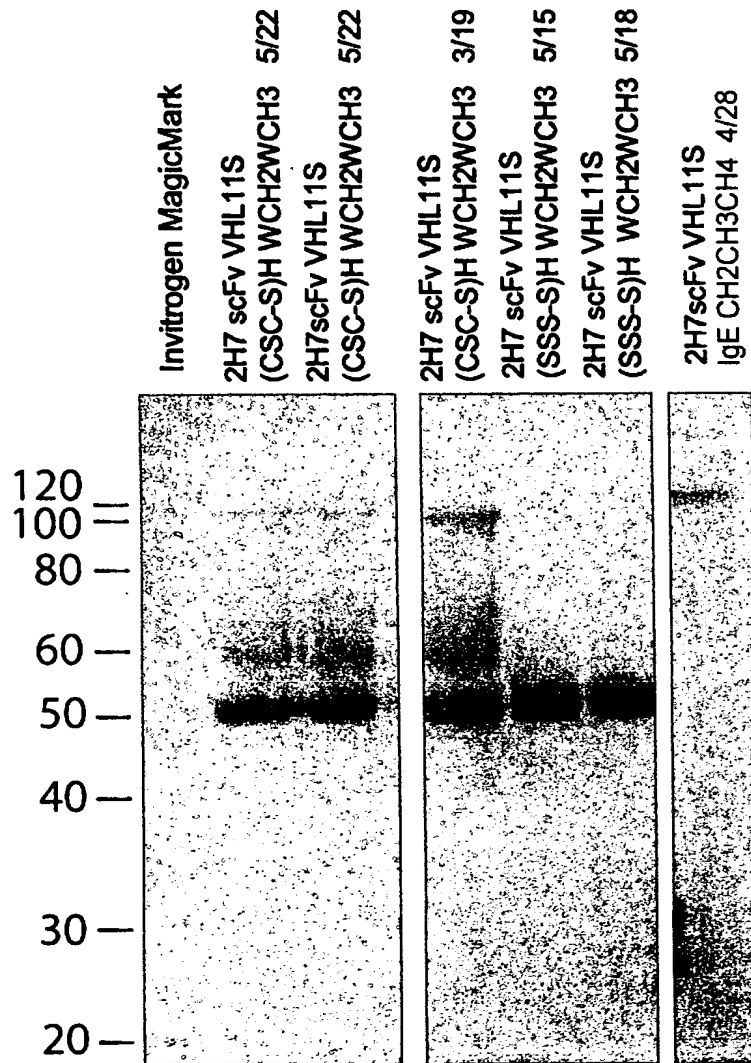
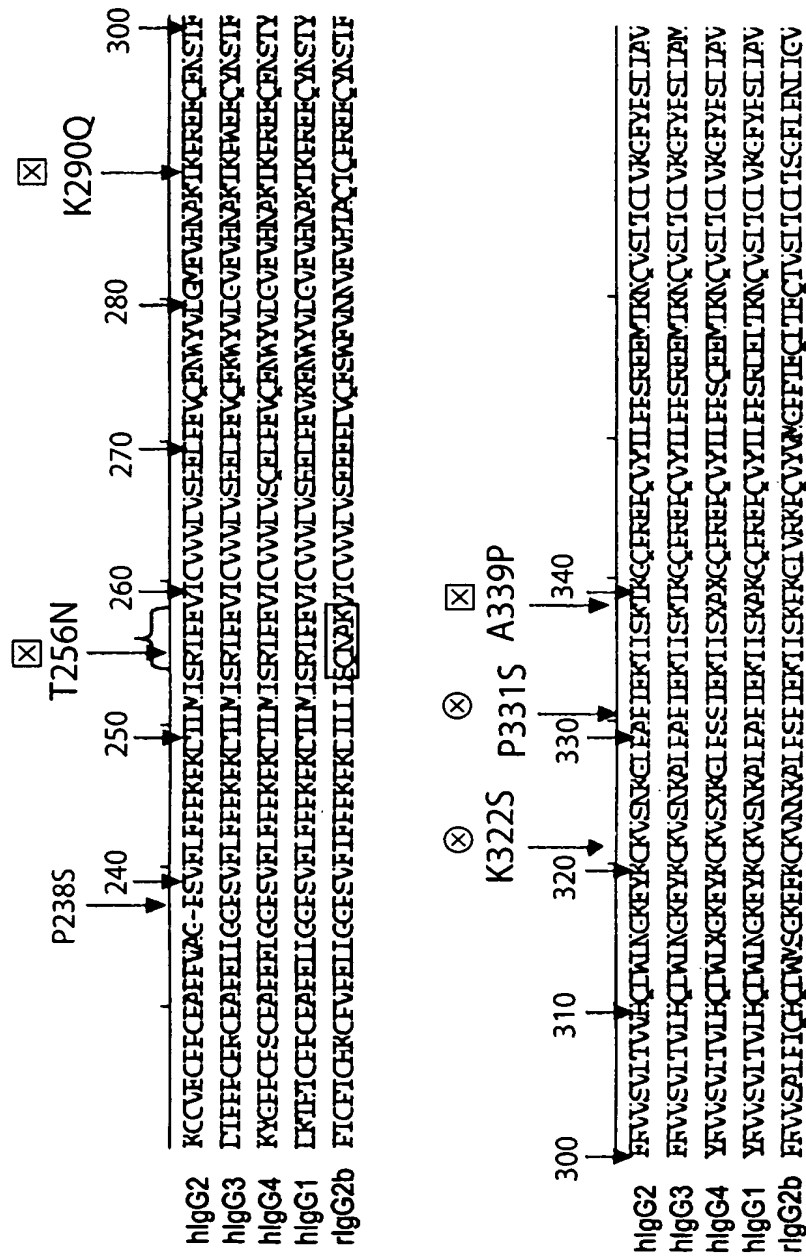


FIG.66

Alterations in Human IgG Fc sequence
that differentially change effector function efficiency



⊗ CDC residue ⊠ ADCC

FIG.67

ADCC Activity of 2H7 scFv VHL11S (CSC-S)H WCH2 WCH3 from
CHO and Lec13-CHO transient transfections

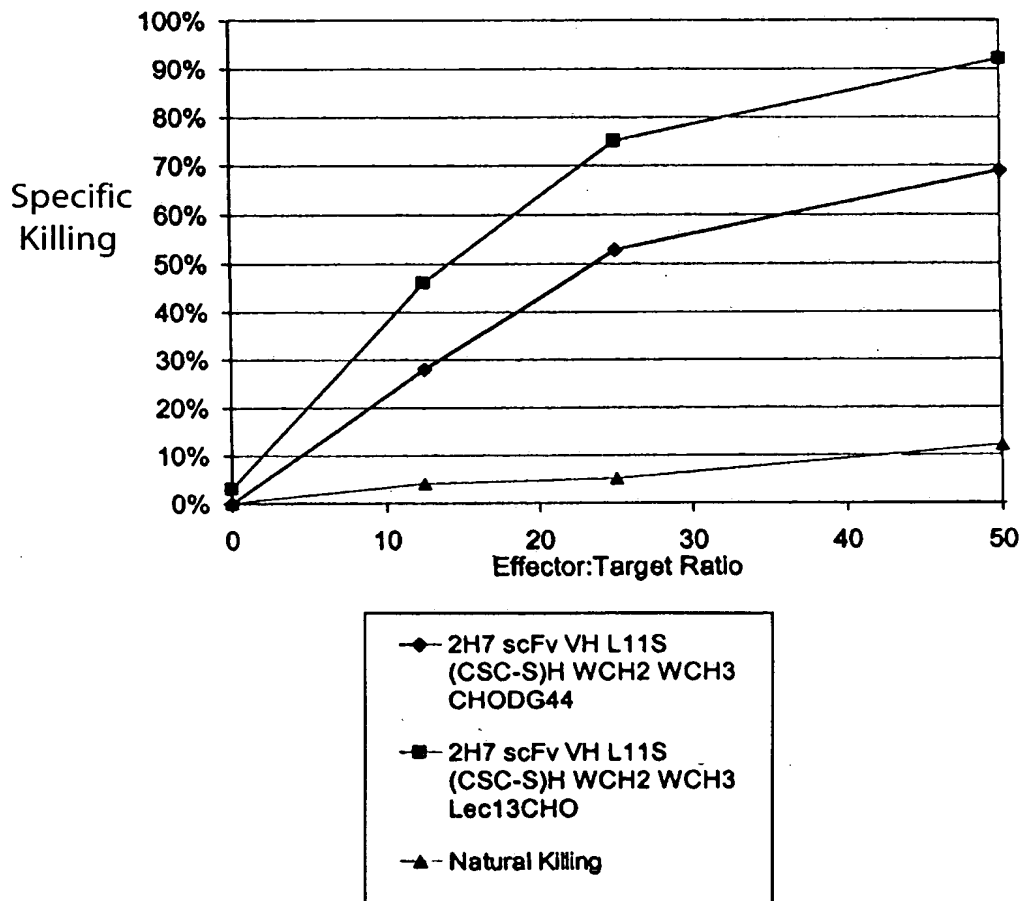


FIG.68

CD16(ED)(SSS-S)H P238S CH2 WCH3 high and low affinity alleles expressed as soluble molecules

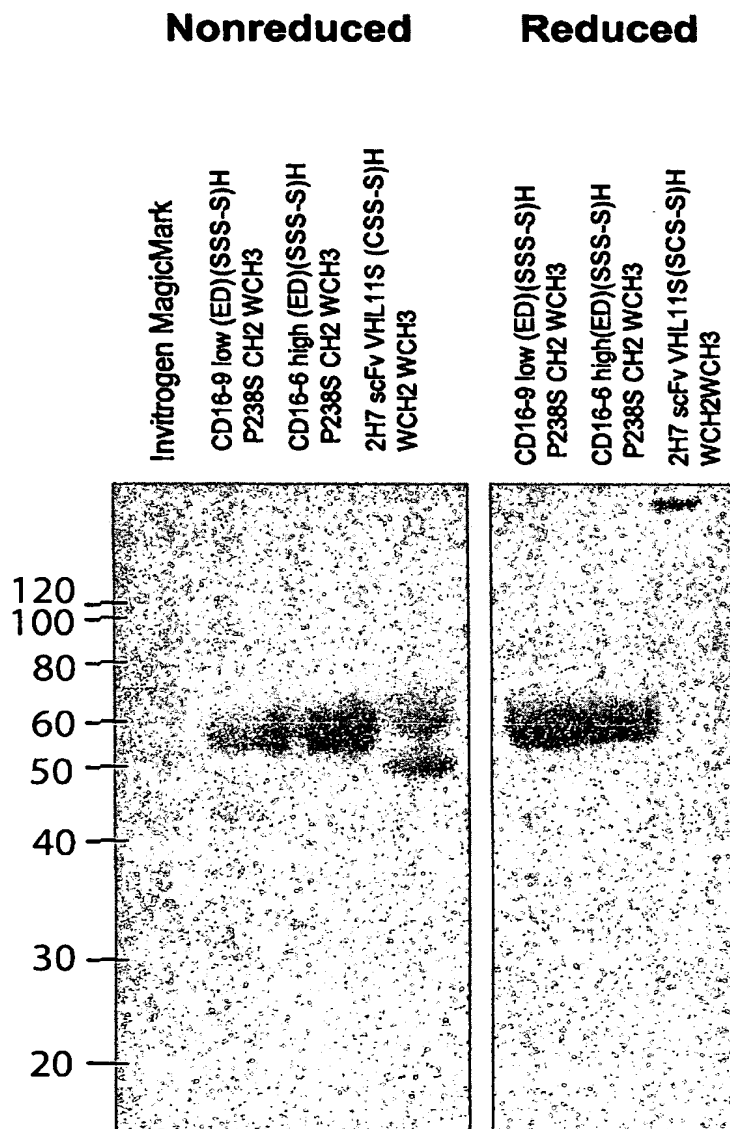


FIG.69

**Binding of soluble CD16-FITC high and low affinity fusion proteins
to 2H7 scFv VHL11S (CSC-S)H WCH2WCH3 or
(SSS-S)H P238S CH2WCH3 on CD20CHO Targets**

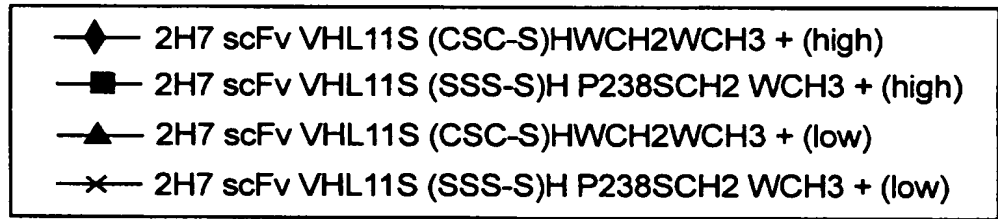
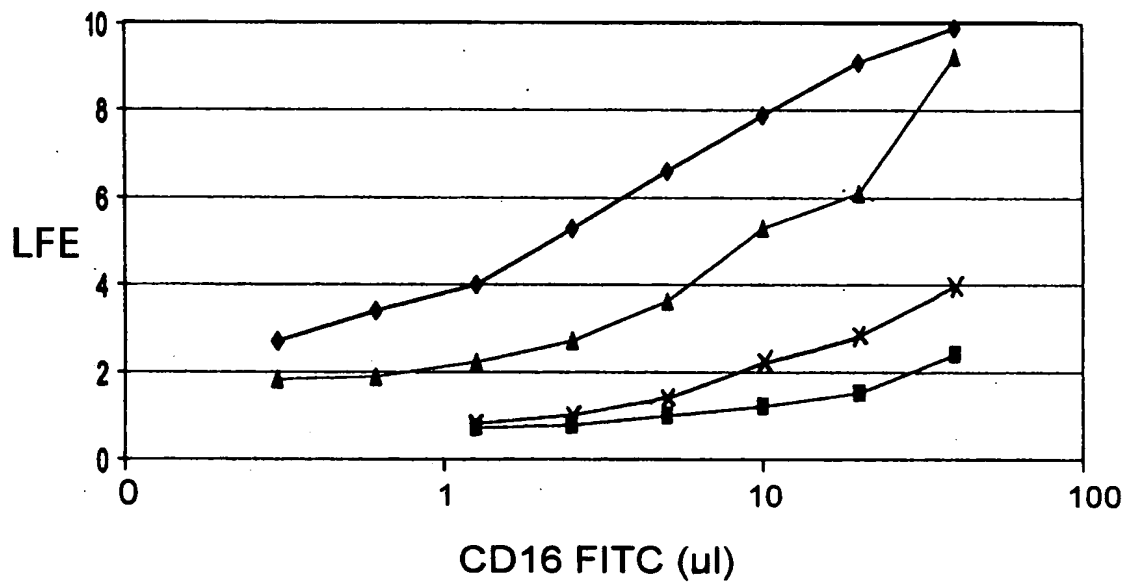
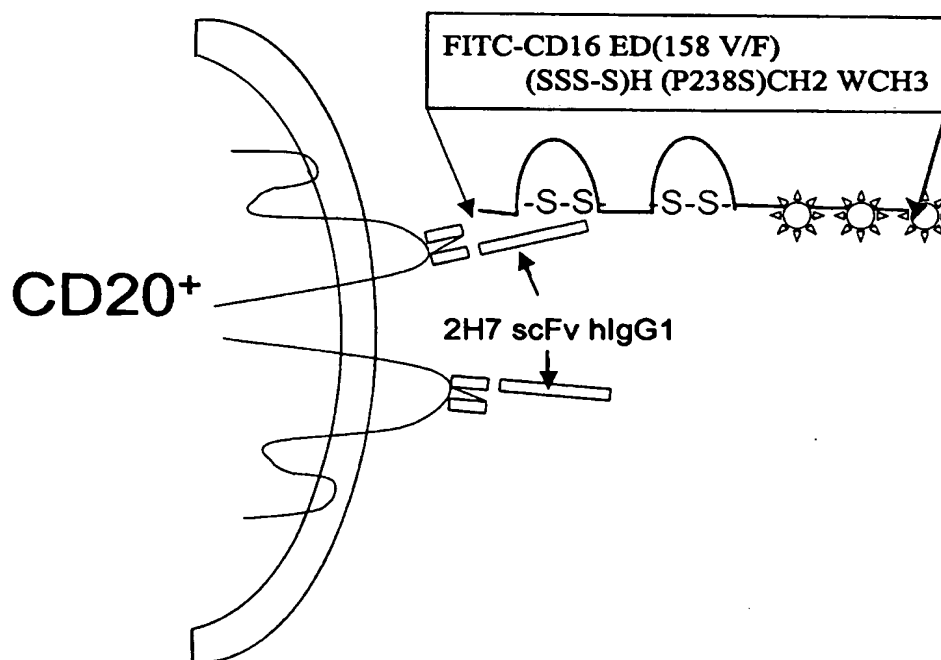


FIG.70

**Binding of FITC Labeled, Recombinant Human
CD16(ED) extracellular domain -Ig Fusion Protein to
CytosB Derivatives on CD20 CHO Cells**



**Expression of surface displayed SMIPs links
modified cDNAs with the altered fusion proteins**

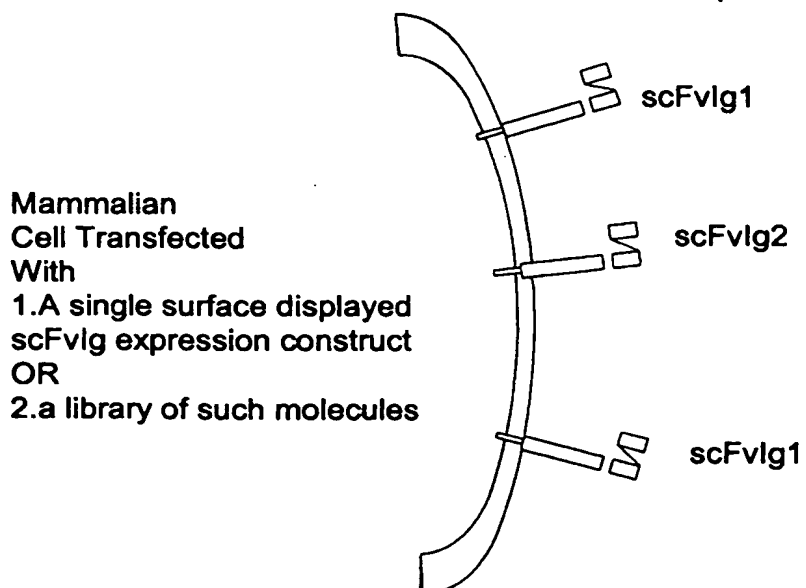


FIG.71

CD37 mAbs and scFvIg Induce Apoptosis

Bjab Staining		Annexin V Positive	
scFvIg	No scFvIg	17.5	
	2H7 MH	27	
	G28-1 MH	30.6	
	G28-1 IgAH	28.9	
	HD37 MH	29.1	
	(2H7+G28-1)MH	41	
	(2H7+HD37) MH	37.1	
	(G28-1+HD37) MH	35.3	
			plus GAM
mAbs	Ramos	AnnexinV Positive	AnnexinV positive
	cells alone	3	3.3
	2H7 Mab	1.4	3.1
	G28-1 Mab	18.3	8.7
	HD37 Mab	3.7	3.1
	G28-5	3.9	8.3
	2H7+G28-1	32.3	35.7
	2H7+HD37	5	10.5
	2H7+G28-5	5.7	19.4
	HD37+G28-1	26.9	50
	HD37+G28-5	8.2	18.4
	G28-1+G28-5	39.5	68.3